AGRICULTURAL RESEARCH INSTITUTE

PUSA

東北帝國大學

理科報告

(生物學)

第一卷

THE

SCIENCE REPORTS

OF THE

TÔHOKU IMPERIAL UNIVERSITY

FOURTH SERIES

(Biology)

SENDAI, JAPAN

Vol. I.

On Sale by
MARUZEN COMPANY, LTD., TOKYO AND SENDAI.

1926

TABLE OF CONTENTS

PAGE

The Biological Institute of the Tohoku Imperial University... *Shinkishi Hatai* 1

Contributions to the physiology of earthworms. II. The effect of temperature on the shortening of the body and the content of water in the body of earthworms. .... *Shinkishi Hatai* 3

Reply to the remarks of Prof. Wilhelm Michaelsen concerning the Perichaeta megascolidioides, Goto and Hatai, and further observations made on this species on the relation of body length to the number of segments and of setae. .... *Shinkishi Hatai* 23

De nova asari specie ex japonia australi. cum Tabula 1. .... *Tokutaro Ito* 45

Metabolic activities of the nervous system. V. 3. On the regular seasonal changes in the relative weight and the sex difference of the central nervous system of Rana nigromaculata. .... *Shigeyuki Komine* 51

Botanische Beobachtungen in Japan .... *Hans Molisch* 75

- **I. Über Elaeoplasten bei Botrychium ternatum Sw. und Ophioglossum vulgatum L.** 75
- **II. Über einen neuen Pflanzenfarbstoff bei Clerodendron trichotomum Thunb.** 79

On the agglutinating substance of the egg of the starfish, Asterina pectinifera. .... *Shichiroku Nomura* 83

Allolobophora foetida (Sav.) in north Japan .... *Kiichiro Sasaki* 89

Zur Kenntnis der Keimentwicklung bei Sargassum. .... *Masato Tahara* 91

Botanische Beobachtungen in Japan .... *Hans Molisch*

- **III. Über das Leuchten des Schlachtviehfleisches in Sendai (Japan).** 97
- **IV. Über das massenhafte Vorkommen von Eiweisspindeln in einer Vaucheria. Mit 1 Tafel.** 105
- **V. Mycoidea parasitica Cunningham, eine parasitische und Phycopeltis epiphyton Millard, eine epiphylle Alge in Japan. Mit 1 Tafel.** 111

VI. Pseudoplasmodium aurantiacum n. g. et n. sp., eine neue Acrasiee aus Japan. Mit 1 Tafel. 119
VII. Über wachsliebende (cerophile) Pilze. Mit 1 Tafel. 123
VIII. Die Eisenorganismen in Japan. Mit 4 Tafeln. 135
IX. Über die Symbiose der beiden Lebermoose Blasia pusilla L. und Cavicularia densa St. mit Nostoc. Mit 2 farbigen Tafeln. 169

Mitosen bei Sargassum. .... *Masato Tahara* und *Naomasa Shimotomai* 189

On the aquatic Oligochaete, Tubifex hattai, N. Sp. .... *Ekitaro Nomura* 193

On the sex ratio in Carassius auratus. .... *Kiichiro Sasaki* 229

On a formula for determining the weight of the brain of Carassius auratus from the weight and length of the entire body, taking into consideration the regular seasonal changes in the relative weight and the difference in sex. .... *Kiichiro Sasaki* 239

Weitere Untersuchungen über die Lebensdauer der Weidensamen. .... *Yôzô Nakajima* 261

Vorläufige Untersuchungen über Myxobakterien in Japan. .... *Yoshiji Yoshii* 277

Effect of light on the movements of the earthworm, Allolobophora foetida (Sav.). .... *Ekitaro Nomura* 293

GRATEFUL ACKNOWLEDGMENT

Owing to the destruction of printing facilities in Tokyo by the earthquake of September 1, 1923, it was necessary to transfer the printing of this series of Science Reports to another press.

The facilities of The Wistar Institute of Anatomy and Biology in Philadelphia, Pennsylvania, U.S.A., for printing and issuing scientific journals were very kindly placed at the service of the Tohoku Imperial University by the President and Board of Managers of that Institute. While this series of Science Reports will be edited and published by the Tohoku Imperial University as usual, it will be issued for a time by The Wistar Institute in Philadelphia.

The Tohoku Imperial University takes this opportunity to express its gratitude to The Wistar Institute for the timely assistance which enables it to continue the publication of this series with the least possible delay.

SCIENCE REPORTS, FOURTH SERIES, BIOLOGY, VOL. 1, NO. 1
AUGUST, 1924

THE BIOLOGICAL INSTITUTE OF THE TOHOKU IMPERIAL UNIVERSITY

In publishing the first biological number of the Science Reports, which appears as Series IV, I wish to announce the formal opening of the Biological Institute of the Tohoku Imperial University.

The erection of the buildings for the Biological Institute was commenced in April, 1921, and has progressed in a satisfactory manner. At the present time, January, 1924, the main building, designed to accommodate investigators in physiology, heredity, and allied biological sciences, and the greenhouse are equipped and in use.

The Marine Biological Station, located at Asamushi, Aomori Ken, is under construction, and it is expected that a portion of the building will be open to biological students and investigators during the summer of 1924.

It is planned to publish complete details of the construction and equipment of the buildings of the Biological Institute when they are finished. The total cost will approximate one million yen.

Although it may be said that the main object of the Biological Institute is to train students in the biological sciences, at the same time the University authorities are keenly aware that the successful school of biology must offer opportunities for the pursuit of research in those sciences.

In the construction of the buildings and in the equipment of the laboratories the main purposes of the Institute and the methods of attaining them have been kept in mind.

The Marine Biological Station will be equipped not only for students, but elaborate provision will be made also for investigators. Among its unique features may be mentioned a specially designed under-sea laboratory provided for observations on experimental evolution and for physiological experiments, and a spacious open marine pool for observation on the growth of marine organisms. In addition, several residences and a large dormitory have been erected where investigators may live with their families and where students will find suitable accommodations.

The Biological Institute has thus been established with the hope that it may prove to be a very considerable factor in the promotion of biological science in Japan. While its success must depend largely upon the activities of its own members, we believe it will be of greatest service to science by cooperating with similar institutions in other parts of our own and in other countries. With this idea in mind, it is my hope that our colleagues may take advantage of the research opportunities we have to offer.

The other Institutes in our Science College have already received proper recognition for their scientific activities. With the hope that the Biological Institute will also merit and receive similar recognition, I take the liberty of adding this brief statement to the first issue of Series IV of the Science Reports.

SHINKISHI HATAI, Ph.D.,
Professor of Zoölogy.

CONTRIBUTIONS TO THE PHYSIOLOGY OF EARTHWORMS

II. THE EFFECT OF TEMPERATURE ON THE SHORTENING OF THE BODY AND THE CONTENT OF WATER IN THE BODY OF EARTHWORMS

SHINKISHI HATAI

FOUR TEXT FIGURES

While studying rhythmic contractions in several species of Oligochaeta (Hatai, '22) it was noted that not only different portions of the body show different forms of rhythms but the forms of the curves grade from simple to complex along the postero-anterior axis. Furthermore, the tissues from which the nerve cord is freed give spontaneous contractions in the tail region, while similarly treated tissues in more anterior parts fail to produce such contraction. These facts suggest that in the earthworm there may exist still other sorts of graded differentiation in other characters than those mentioned in the above and I have tested this question from the standpoint of the heat effect.

The technique employed for the present investigation is essentially the same as that used in connection with my former investigation (Hatai, '22), but in this case the utmost care was taken to determine accurately the degree of the temperature at which the tissue showed the initial heat rigor. For such determination it was found most important to use active and fresh specimens only, since such tissues alone can produce sudden contractions as the result of the gradual rise of temperature, while older and less active specimens produce generally very gradual contractions and hence the point of contraction cannot be determined with certainty.

In the present investigation I shall discuss only the point at which the tissue showed initial heat rigor; the temperature at which the maximum contraction occurs as well as the temperature at which the second rise of heat rigor appears will not be considered.

For the materials of the investigation I have chiefly employed the earthworm, Perichaeta megascolidioides, Goto and Hatai, on account of the greater size of its body as well as the greater elasticity of the tissue. The other worms which were used for the purpose of comparison were Per. divergens, Mcls., and several forms of marine annelids.

In order to distinguish the different portions of the body I have adopted the following designations:

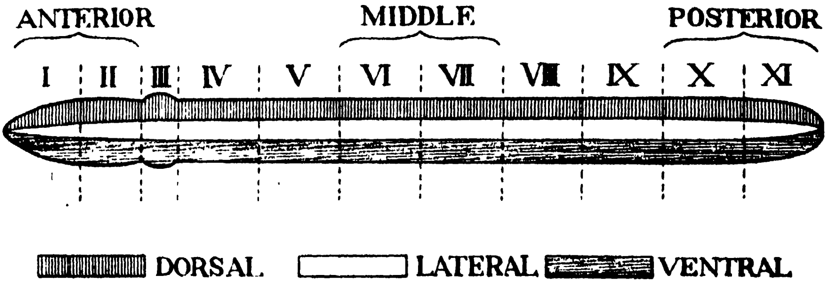


Diagram 1

The preclitellar portion, usually called the head, was divided into two portions, and the foremost including the mouth designated I and the other designated II. The clitellum itself is designated III. The postclitellar portion of the body was divided into eight equal portions and these portions were designated according to the natural order of segments which were taken; that is, the portion next to the clitellum is designated IV and the segment which follows immediately caudad to IV was designated V, and finally the tail end was designated XI. Diagram 1 illustrates the statements given above. It must be added that in some instances I have also designated the preclitellar region 'anterior,' the last two caudal divisions X and XI 'posterior,' and the divisions which were numbered VI and VII were designated 'middle.'

In most cases the body wall alone was used, but when the worms were either too small for such operation or the tissues broke too easily, the entire segments together with the internal organs were used.

In several instances I have further divided the body into three divisions, the dorsal, the lateral and the ventral, and tested each separately.

The color of the dorsal region is deep pinkish brown, while that of the ventral region is white, but toward the lateral midline the colors are gradually mixed. I have therefore divided the ring into three nearly equal areas without regard to the colors, as is shown in diagram 2, and designated those divisions as dorsal, lateral and ventral, as illustrated in the figure; though such treatment is arbitrary, nevertheless physiological reactions are definitely distinguishable from each other as will be seen in subsequent pages. Lastly, for the determination of a point which corresponds to the onset of heat contraction, I have adopted the following simple device. With a sharply pointed hard pencil a line was drawn parallel to the base line (or in this case the line drawn by the temperature recording lever), closely touching the curve. The point of divergence which was formed by the straight line thus drawn and the curve traced by the contraction of the tissue was taken as the point of onset of the heat contraction. As has been stated above, unless the tissue is fresh and active the point of divergence cannot be determined with accuracy owing to the very gradual rise, and consequently there is a failure to show a sudden change of direction.

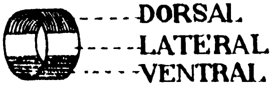


Diagram 2

THE EFFECT OF HEAT ON DIFFERENT PORTIONS OF THE BODY

Vernon ('99) found in Lumbricus that the anterior part requires the highest temperatures for the production of the initial contraction when compared with both the middle and posterior parts. The middle part differs in this respect very little from the posterior, but the former tends to require a slightly higher temperature than the latter. Thus, according to Vernon, the temperature grades from the head towards the tail, the former requiring the highest while the tail region requires the lowest. The significance of this peculiar behavior of the tissues toward heat will be discussed later and I shall first present data which I have gathered from the Japanese species of earthworms.

As a preliminary I have made some tests, using Perichaeta divergens of Michaelsen, and obtained the following results:

Perichaeta divergens (no. 1)

	Onset of initial contraction, °C.
Anterior	40.7
Middle	36.8
Posterior	38.6

In this instance, entire portions of the body together with the organs contained in the segment were used, and thus the present test is directly comparable with the observations of Vernon. We find as Vernon found in Lumbricus that the anterior segments require much higher temperature when compared with the rest of the body, but contrary to Lumbricus the tail end requires definitely higher temperature than the middle. This we found to be generally true even when the body wall alone was tested without the internal organs. The following table shows this clearly:

Perichaeta divergens (no. 2)

	Dorsal	*Ventral*	*Average of dorsal + ventral*
	Degrees	Degrees	Degrees
Anterior	40.0	38.7	39.4
Middle	37.9	37.2	37.6
Posterior	37.8	38.7	38.3
Average	38.6	38.2	

Perichaeta megascolidioides (*no. 1*)

	Dorsal Degrees	*Ventral* Degrees	*Average of dorsal + ventral* Degrees
Anterior	41.3	38.5	39.9
Middle	39.2	38.0	38.6
Posterior	40.7	37.0	37.9
Average	40.4	37.8	38.9

Perichaeta megascolidioides (*no. 4*)

	Dorsal Degrees	*Ventral* Degrees	*Average of dorsal + ventral* Degrees
Anterior	42.8	40.4	41.6
Middle	38.2	36.7	37.5
Posterior	37.3	36.2	36.8
Average	39.4	37.8	

This specimen was large, but the clitellum was not yet developed.

From these three examples we notice clearly that the anterior portion of the body requires the highest temperature to produce initial heat rigor when compared with the other portions of the body. As was noted by Vernon, the middle portion differs but very little from the posterior region, but in our material the posterior tends to require a shade higher temperature than the middle. I may add that the difference just stated in these two portions of the body is often very distinct as will be seen from numerous other examples given in subsequent pages.

The above example brought out one more point which was not at all anticipated; that is, the dorsal region requires in almost all cases definitely higher temperature to produce the initial heat rigor than the ventral region. We further notice that the temperature differences between the dorsal and ventral are greater than the temperature differences between the middle and posterior parts of the body.

Before discussing the results I shall present further data in which the determination was made on more than three portions of the body.

Perichaeta megascolidioides (*no. 3*). *Adult*

	Dorsal	*Ventral*	*Average of dorsal + ventral*
	Degrees	Degrees	Degrees
Anterior (I)	43.2	41.5	42.4
Middle (VI)	41.6	38.9	40.3
Middle (VII)	40.7	38.7	39.7
Posterior (X)	43.5	39.0	41.3
Posterior (XI)	40.7	38.8	39.8
Average	41.9	39.4	

Perichaeta megascolidioides (*no. 4*). *Adult*

	Number of segments	*Dorsal*	*Ventral*	*Average of dorsal + ventral*
		Degrees	Degrees	Degrees
Anterior (I)	6	41.3	41.0	41.2
(II)	7	41.5	40.9	41.2
Clitellum (III)	6	41.2	39.8	40.5
Middle (IV)	10	40.4	39.0	39.7
(V)	11	39.6	39.2	39.4
(VI)	15	39.4	38.7	39.1
(VII)	13	39.3	38.7	39.0
(VIII)	14	39.2	38.7	39.0
(IX)	10	39.8	37.9	38.9
Posterior (X)	10	40.0	37.8	38.9
(XI)	9	40.9	37.8	39.4
Average		40.2	39.4	

These two examples show clearly that the general statements so far made still hold true. Here we further notice that the amount of heat which is necessary to produce the initial heat rigor decreases gradually from the anterior toward the posterior but reaches the minimum at about or near the end of the middle body (VII and VIII); however, it rises again and at the tail end reaches a second maximum.

I may point out that in the posterior two portions (nos. X and XI), X sometimes requires higher temperature and XI in other cases, and I am thus far unable to state definitely which one of the two portions ought to be considered the second maximum.

So far I have tested the effect of heat on adult earthworms, but whether or not this phenomenon of 'heat shortening gradient' is peculiar to the adult worms only would be very interesting to determine. Unfortunately, however, I had in my laboratory only one young specimen, measuring about 1½ inches when contracted, to test, but I have decided to present the data below as a matter of interest.

Perichaeta megascolidioides (*no. 5*). *Very young, small specimen*

	Order of samples	*Dorsal*	*Ventral*	*Average of dorsal + ventral*
		Degrees	Degrees	Degrees
Anterior (mouth)	1	43.5	38.5	41.0
	2	38.6	38.4	38.5
	3	42.8	38.7	40.8
	1	42.4	39.4	40.9
Posterior (anus)	5	42.9	37.8	40.3
Average		42.0	38.6	

In this specimen the entire body was cut into five equal pieces and the test was made of the parts together with the internal organs. Although a single observation, nevertheless a close agreement of the results with all adult specimens suggests strongly that the 'heat shortening gradient' is already present in this stage of growth. We must therefore await further opportunity to make additional observations on more immature individuals to decide the above question.

In subsequent pages further data are given with the hope. 1) of determining the extent of individual variations and, 2) to present further additional observations on the lateral regions of the body.

Perichaeta megascolidioides (*no. 6*). *Adult, but small*

	Dorsal	*Lateral*	*Ventral*	*Average of dorsal + lateral + ventral*
	Degrees	Degrees	Degrees	Degrees
Anterior	40.0	39.6	36.1	38.6
Middle	35.0	36.8	36.8	36.2
Posterior	38.0	37.7	38.2	38.0
Average	37.7	38.0	37.0	

Perichaeta megascolidioides (*no. 7*). *Adult*

	Dorsal	*Lateral*	*Ventral*	*Average of dorsal + lateral + ventral*
	Degrees	Degrees	Degrees	Degrees
Anterior	44.3	44.4	43.0	43.9
Clitellum	42.7	40.6	41.0	41.4
Middle	40.4	41.4	39.8	49.5
Posterior (X)	40.0	38.8	38.5	39.1
Posterior (XI)	41.0	38.0	39.5	39.5
Average	41.7	40.6	40.4	

Perichaeta megascolidioides (no. 8). Adult, large

		Dorsal	*Lateral*	*Ventral*	*Average of dorsal + lateral + ventral*
		Degrees	Degrees	Degrees	Degrees
Anterior	(I)	43.9	41.5	42.0	**42.5**
Anterior	(II)	43.4	43.2	42.2	**42.9**
Middle	(VI)	40.7	39.8	39.8	**40.1**
Middle	(VII)	39.0	39.6	39.0	**39.2**
Posterior	(X)	41.0	39.6	39.0	**39.9**
Posterior	(XI)	41.9	41.9	41.5	**41.8**
Average		41.7	40.9	40.6	

Perichaeta megascolidioides (no. 9). Adult, large

		Dorsal	*Lateral*	*Ventral*	*Average of dorsal + lateral + ventral*
		Degrees	Degrees	Degrees	Degrees
Anterior	(I)	41.4	41.3	—	-
Anterior	(II)	41.6	39.7	39.9	**40.4**
Middle	(VI)	39.0	38.7	38.6	**38.8**
Middle	(VII)	36.6	36.5	37.5	**36.9**
Posterior	(X)	42.0	38.6	37.0	**39.2**
Posterior	(XI)	40.1	37.6	36.0	**37.9**
Average		39.9	38.2	37.8	

X not included in the average.

Perichaeta megascolidioides (no. 10). Adult

		Dorsal	*Lateral*	*Ventral*	*Average of dorsal + lateral + ventral*
		Degrees	Degrees	Degrees	Degrees
Anterior		43.0	43.5	42.1	42.9
Middle	(VI)	40.0	39.0	40.5	39.8
Middle	(VII)	41.1	40.8	41.3	41.1
Posterior		40.8	39.7	39.9	40.1
Average		41.2	40.8	41.0	

The data taken from the five worms above show again that, with few isolated exceptions, the anterior region requires the highest temperature to produce heat rigor while the posterior surpasses the middle in this respect and the dorsal region requires distinctly higher temperature than the ventral.

The lateral region tends to show a greater degree of variability than in any other instances so far found, but the existence of a strong tendency to require much higher temperature than in the ventral and lower temperature than in the dorsal region cannot be denied: in other words, the lateral stands between the dorsal and ventral. The greater variability which was found in the lateral region was due probably to the technical difficulty of separating the lateral region from both the dorsal and the ventral. It must be admitted that in dividing the rings into three regions sometimes more of the dorsal tissue is included with the lateral and sometimes more of the ventral. On account of this difficulty of technique the occurrence of somewhat larger irregularity cannot be avoided.

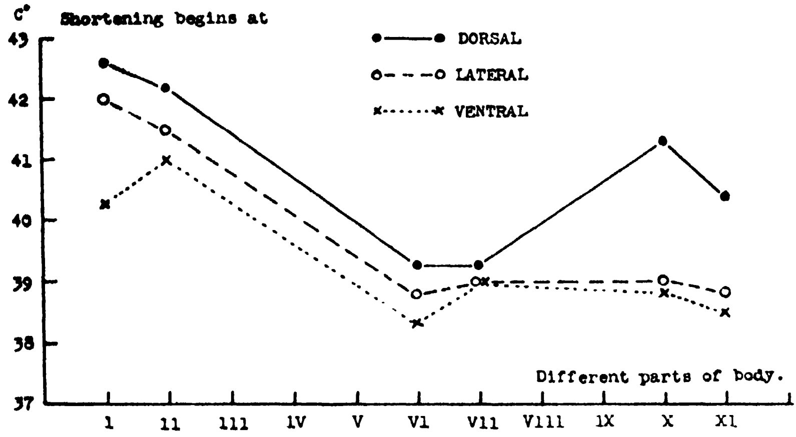


Chart 1 The initial onset of 'heat rigor' on the different portions of the body of the earthworm, Perichaeta megascolidioides, Goto and Hatai.

In order to bring out more clearly various relations so far presented I have given in table 1 the averages of all cases which are contained in the above tables together with a graph based on these averages.

From the combined data, as well as from the graphic illustration (chart 1), it is clear that the initial onset of the heat shortening is considerably different according to the different portions taken. Generally speaking, the amount of temperature required in the different regions diminishes from the anterior head end caudad and reaches the minimum at about the middle portion of the body; in turn it again rises toward the tail and reaches a second maximum at the anal region.

Both the lateral and ventral body walls behave similarly to the dorsal body wall. As may be noticed, there is another axis of the gradient from the dorsal toward the ventral; that is, the amount of temperature required gradually diminishes along the dorsoventral axis.

OBSERVATIONS ON THE MARINE ANNELIDS

During the month of August, 1922, I had opportunity to carry on a similar investigation at Asamushi, a small mineral hot-spring town along Awomori Bay. To my regret, however, the facilities for such investigation were very limited and the results were not entirely satisfactory; neverthless I have decided to present these for the purpose of comparison:

Nereis yesoensis

		No. 1	*No. 2*	*No. 3*	
				Dorsal	*Ventral*
		Degrees	Degrees	Degrees	Degrees
Anterior		47.2	—	48.0	47.5
Middle	I.	45.1	47.0	47.5	47.0
Middle	II.	45.6	—	48.0	46.7
Middle	III.	45.6	—	—	—
Posterior		46.0	47.8	—	—

Ceratocephale osawai

		No. 1	*No. 2*	*No. 3*	*No. 4*	*No. 5*	
						Dorsal	*Ventral*
		Degrees	Degrees	Degrees	Degrees	Degrees	Degrees
Anterior		47.3	45.2	49.2	48.5	47.6	47.6
Middle	I.	43.5	46.5	47.0	—	46.0	46.2
Middle	II.	43.2	—	47.0	—	45.8	45.9
Middle	III.	—	—	43.5	—	44.7	45.4
Posterior		43.7	46.5	—	48.0	—	—

The observations made on these two species of marine annelids suggest that, so far as the present data are concerned, the anterior head region requires a much higher temperature than either the middle or posterior and there is also tendency for the posterior tail portion to require a shade higher temperature than the middle portion of the body. As to the dorsoventral gradient, the dorsal requires a higher temperature than the ventral in Nereis yesoensis, but this is indeterminable with certainty in the case of Ceratocephale osawai.

TABLE 1

Perichaeta megascolidioides, Goto and Hatai

ANTERIOR (I)			ANTERIOR (II)			MIDDLE (VI)			MIDDLE (VII)			POSTERIOR (X)			POSTERIOR (XI)		
Dorsal	Lateral	Ventral	Dorsal	Lateral	Ventral	Dorsal	Lateral	Ventral	Dorsal	Lateral	Ventral	Dorsal	Lateral	Ventral	Dorsal	Lateral	Ventral
Degrees	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*
44.3	44.4	43.0				40.4	41.4	39.8				46.0	38.8	38.5	41.0	38.0	39.5
40.0	39.6	36.1				35.0	36.8	36.8							38.0	37.7	38.2
43.9	41.5	42.0	43.4	43.2	42.2	40.7	39.8	39.8	39.0	39.6	39.0	41.0	39.6	39.0	41.9	41.9	41.5
		39.6						35.2									41.3
43.0	43.5	42.1				40.0	39.0	40.5	41.1	40.8	41.3				40.8	39.7	39.9
			41.6	39.7	39.9	39.0	38.7	38.6	36.6	36.5	37.5	42.0	38.6	37.0	40.1	37.6	36.0
41.3		38.5				39.2		38.0							40.7		37.0
43.2		41.5				41.6		38.9	40.7		38.7	43.5		39.0	40.7		38.8
42.8	41.0	40.4				38.2	37.1	36.7							37.3		36.2
41.3		41.0	41.5		40.9	39.4		38.7	39.3		38.7	40.0		37.8	40.9		37.8
43.5		38.5													42.9		37.8
42.6	*42.0*	*40.3*	*42.2*	*41.5*	*41.0*	*39.3*	*38.8*	*38.3*	*39.3*	*39.0*	*39.0*	*41.3*	*39.0*	*38.8*	*40.4*	*38.8*	*38.5*

It seems reasonable, however, to conclude that so far as the heat-shortening gradient along the longitudinal axis of the body is concerned these two marine species behave similarly to the terricola species of annelids, the earthworms.

In this connection I refer with great interest to the observations on the chaetopod annelids by Hyman and Galigher in regard to physiological activity ('21). These investigators found that in both Nereis virens and Nereis vexillosa the metabolic rate is high at the anterior and posterior ends and decreases from both ends to the middle region. Approximately similar relations were found in the oxygen consumption of pieces from different levels. The possibility of causal connection between the metabolic rate and the heat-shortening gradient is discussed in the subsequent pages.

I have made additional observations on another species of marine annelids, Laonome japonicus. This species, which lives in the chitinous tube, behaves differently from all other species so far examined, and I shall now present the results of my observations.

Laonome japonicus

	No. 1	*No. 2*	*No. 3*	*No. 4*		*No. 5*	
				Dorsal	*Ventral*	*Dorsal*	*Ventral*
	Degrees	Degrees	Degrees	Degrees	Degrees	Degrees	Degrees
Anterior	40.7	42.3	41.9	40.0	40.1	40.0	41.3
Middle I.	40.5	41.3	40.3	—	40.0	41.0	41.0
Middle II.	39.7	40.2	—	38.5	38.5	41.0	—
Posterior	38.3	38.8	38.5	40.0	40.1	39.4	38.5

In Laonome japonicus the phenomenon of the gradient is very simple; that is, the amount of heat which is necessary to produce onset of the heat shortening gradually diminishes along the longitudinal axis of the body cephalocaudally. Furthermore, the dorsoventral differentiation, which is clearly demonstrable in earthworms, is totally absent. The significance of this unique phenomenon of 'heat shortening gradient' in this species is not clear, but we know that the laonomes pass a sedentary life within the tube while the other species of the annelids under consideration move about freely. We are thus tempted to connect the difference in the phenomenon of gradients with the difference in their habitat. Using this as a working hypothesis, it is the wish of the writer to continue further investigation in the future.

PERCENTAGE OF WATER IN DIFFERENT PORTIONS OF THE BODY

With the hope that the temperature at which the heat shortening is normally produced might be altered by altering the concentration of the liquid medium in which the tissue is placed, I have first attempted to determine the content of the water in tissues taken from different portions of the body of the earthworms.

For this purpose the body walls alone were used and the alimentary tract and nervous system were always removed. The water content was determined by keeping the tissue for one week in a hot-water oven with temperature records between 95° and 100°. The body fluid, which usually adheres to the tissue during dissection, was removed by gently pressing the tissue between blotting-papers. The water content was estimated in both Perichaeta divergens and P. megascolidiodes, and the results are shown in table 2.

TABLE 2

NUMBER OF EARTHWORMS	PERCENTAGE OF WATER										
	Anterior		Clitellum	Middle						Posterior	
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
					Perichaeta divergens, Mels.						
3	77.8	77.3	73.9	78.3	79.8	81.0	81.3	82.1	81.8	81.1	80.3
4	79.1	78.6	73.0	81.6	81.4	82.3	81.5	1.3	80.8	81.7	80.5
5	79.1	81.2	75.6	80.9	81.6	82.3	82.0	82.1	81.6	81.7	79.8
6	79.5	80.4	81.9	83.3	83.7	83.7	83.5	83.9	83.1	83.3	81.0
Average	*78.9*	*79.4*	*76.1*	*81.0*	*81.6*	*82.3*	*82.2*	*82.4*	*81.8*	*82.0*	*80.4*
					Perirchaeta megascolidiodes, Goto and Hatai						
11	78.9	79.1	74.8	80.4	81.7	81.6	82.3	82.3	81.9	81.7	81.0
12	79.4	79.8				83.4	83.7			83.5	82.3

The percentage of water found in different portions of the body varies, though the amount of variation is not very large. The greatest difference compared with the other portions of the body is noted in the clitellum. This, however, is to be expected since the clitellum is largely glandular in nature and its secretory activity varies according to the season as well as to the age of the individuals. We are familiar with the fact that under laboratory conditions some individuals show glandular activity as can be judged from the color and degree of swelling, while in others the clitellum becomes atrophied and semidried. The water content under such different conditions ought to show larger differences. Such was the case with the samples taken in the present investigation and we notice that one individual (no. 4) gives a high percentage while the rest of the worms give a low degree of water content.

We notice that the percentage of water is closely similar in these two species of earthworms, and furthermore the similarity becomes more evident when corresponding portions of the body are compared.

Perhaps one of the most significant points which was revealed from the present investigation is that the water content is least in the anterior head end and greatest in the middle portions of the body.

Generally speaking, the percentage of water increases anteroposteriorly and reaches the first maximum in the middle of the body, but again decreases and reaches a second minimum in the posterior anal region.

The percentage of water then shows exactly reverse relation to the amount of heat which is necessary to produce the initial onset of the heat shortening in the corresponding portions of the body.

So far as the present observations are concerned, we conclude that the water content is least in the tissue where the highest temperature is needed to produce the initial onset of heat shortening, and it is greatest where the lowest temperature is required for the same purpose.

Whether or not these reverse relations just pointed out indicate causal connections between these two phenomena or whether these relations are mere coincidences cannot be decided definitely. However, from the facts that the heat shortening may be altered by adding different amounts as well as different kinds of salts, as the experiments of Moore ('02), of Vernon ('99) and of Meigs ('09) have shown, the intimate relation between the water content and the heat shortening which was found in the earthworm seems highly probable. I have shown these reverse relations in chart 1 based on the data which were given in table 2 and have also shown one case of the heat shortening which was taken from P. megascolidioides (no. 4) for the purpose of comparison.

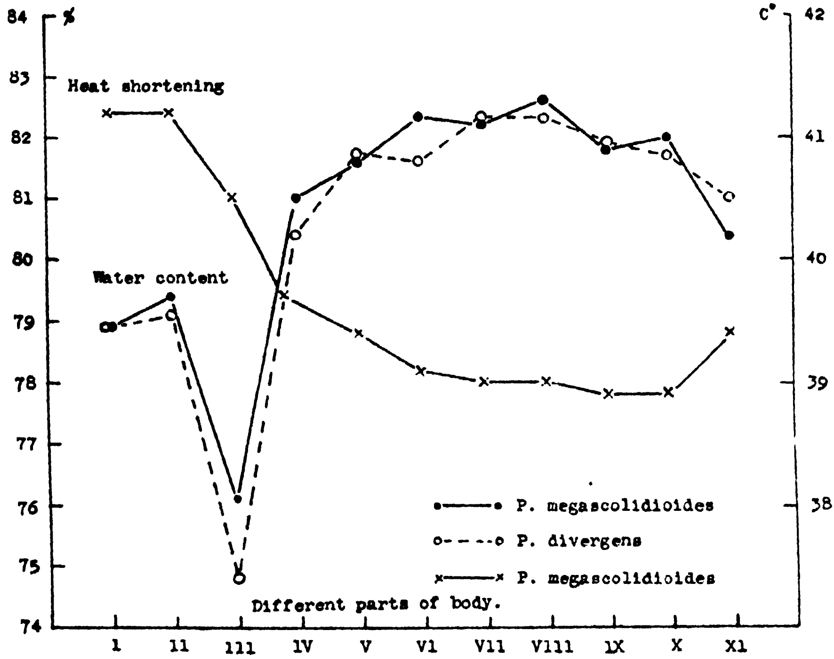


Chart 2

In this connection the observation of Vernon is very interesting and I shall therefore allude to his experiment a little more in detail.

With a view to determining how far the temperatures of their onset and culmination are capable of being influenced by external agencies, Vernon kept the sartorius and gastrocnemius of R. temporaria in various strengths of saline solutions for two or three hours previous to testing the tissues with a gradually rising heat, and found that the initial heat contraction was considerably affected. "Thus in 0.6% solutions the temperature of onset was generally about 30°, but after keeping in 0.2 to 0.4% salt solutions, it fell to only a little above 20°. The hyperisotonic solutions, on the other hand, generally raised it several degrees above 30°, or in the case of the 4% solution, even to 45°." In another series of experiments Vernon kept the gastrocnemius in the saline solutions for from twenty-two to twenty-four hours previously. The muscles were heated in 0.6 per cent saline instead of the saline in which they had been kept. Vernon found that the temperature of onset of the initial heat contraction in the hypoisotonic solution is 5.6° lower than in the isotonic, while in the hyperisotonic solution it is 4.5° higher.

From the effects described above of soaking muscles in various saline solutions, Vernon thought that the temperature of heat contraction and of loss of excitability of the tissues might depend largely on the amount of solid constituents they contained. After actual tests Vernon came to the following conclusion: "Contrary to expectation, the temperatures of onset of heat contraction and of loss of excitability do not seem to depend at all on the amount of solid constituents in the tissues. Thus the tissues of the grass snake lose their excitability at 42.5°, and contain 25% of solids, but those of the tortoises lose it at about 46°, and contain only 18%. Those of the eel lose it at the same temperature as those of the goldfish, but contain 36% of solids as compared with 15%, etc. As a rule, the involuntary muscles contained nearly as much solids as the voluntary, but the cardiac muscles about 3% less."

These observations of Vernon are certainly interesting, but unfortunately he did not determine the solid content of muscles belonging to the same animal in which the amount of heat needed to produce onset of initial heat contraction differed: for instance the gastrocnemius of R. temporaria according to Vernon requires 33.3° while the pectoralis major of the same animal requires but 28°, and therefore if these two kinds of muscles just mentioned were compared with respect to the solid constituents and no difference were found then this conclusion would have been more convincing. However, Vernon compared the solid constituents of animals belonging to very widely different animal classes instead of comparing the tissues belonging to the same animal, hence his statement cannot be accepted in entirety.

At least my own observations on the earthworm force me to a contrary conclusion to that of Vernon; that is, that the temperature of the onset of heat contraction is proportional to the amount of solid constituents which are contained in the tissues.

GENERAL REMARKS

The preceding data show clearly that in the earthworm the amount of heat which is necessary to produce the initial onset of heat shortening is different according to different portions of the body. The anterior head region requires the highest, the posterior tail region stands next, while the middle portion requires the least amount of heat. A similar gradation is shown along the dorsoventral axis; that is, the dorsal portion requires the greatest amount of heat, the ventral the lowest, while the lateral portion stands between those two. The gradations are regular and orderly and are true not only in Perichaeta megascolidioides but in several other annelids so far examined with the exception of Laonome japonicus, in which species the gradients continuously diminish caudalward along the longitudinal axis of body. The gradations along the dorsoventral axis were always clear in some of the annelids as has been stated already.

The content of water in the tissue behaves similarly to what we have found in regard to the heat reaction, and where the greatest amount of heat is required to produce initial heat contraction then it contains the least amount of water or the greatest amount of solids and vice versa. Thus we may conclude that in the earthworms under consideration the amount of heat which is necessary to produce initial onset of heat shortening is directly proportional to the amount of solid constituents contained in the tissue.

The true significance of these various phenomena cannot be fully revealed until a great many other related facts are investigated. One naturally inclines to interpret the facts by an interesting hypothesis of Child, the 'metabolic gradients.' However, I have as yet no direct evidence to prove that the present phenomenon is causally connected with the metabolic activity of the animals. If, however, the so-called initial heat contraction was the result of the formation of lactic acid, we may correlate this heat shortening gradient with so-called susceptibility gradients of Child.

Taylor and Alvarez ('17) found in excised segments from different parts of a rabbit intestine that the rhythmic contraction is accelerated with a temperature rise. This rate is greater for the segments from the lower bowel than for those taken from the upper bowel. These investigators conclude that the rate of rhythmic contraction is determined by the chemical changes taking place in the muscle. It seems conceivable then that one part of the annelid body which undergoes heat shortening faster than other parts might be due to greater chemical changes which are taking place in one part than in the other, or in other words, the heat shortening gradient is an outward expression of corresponding degree of physiological activity.

Recently Hyman ('16) found that in Aeolosoma the metabolic gradient is of the primary type with some indications of a posterior region of high metabolic rate. But in Lumbriculus the high metabolic rate is found both in anterior and in posterior portions of the body. Further findings of Hyman and her collaborators on the correlation between metabolic gradients and electrical responses in annelids suggest strongly that the heat-shortening gradient which was noted is causally connected with the physiological activity of the animal since any outward expression of the functional activity must accompany some sort of morphological or structural gradients.

At any rate we must still seek more direct evidence that the gradients which were found in the present investigation are related to the gradient in physiological activity of the earthworm before the theory of metabolic gradients can be applied to the present findings.

We, however, note with interest at the present moment that the body of the earthworm shows not only a high degree of morphological differentiation, but as to the solid constituents as well as to the effect of heat the tissues show an equally high degree of differentiation. Since further investigation is actively going on with a view to determine the functional gradients of different portions of the body, I reserve a final interpretation of the facts which were presented in this paper to the near future.

LITERATURE CITED

Child, C. M. 1913 Studies, etc. VI. The nature of the axial gradients in Planaria and their relation to antero-posterior dominance, polarity, and symmetry. Arch. f. Entw.-mech., Bd. 35, S. 598–641.

1915 Individuality in organisms. The University of Chicago Press.

1921 The origin and development of the nervous system. The University of Chicago Press.

Hatai, Shinkishi 1922 Contributions to the physiology of earthworms. I. The effect of heat on rhythmic contractions in several species of Oligochaeta. Japanese Journal of Zoölogy, vol. 1, no. 1.

Hyman, Libbie H. 1916 An analysis of the process of regeneration in certain Microdrilous Oligochaetes. Jour. Exp. Zoöl vol. 20, no. 2.

Hyman, L. H., and Galigher, A. E. 1921 Direct demonstration of existence of a metabolic gradient in annelids. Jour. Exp. Zoöl., vol. 34, no. 1.

Hyman, L. H., and Bellamy, A. N. 1922 Studies on the correlation between metabolic gradients, electrical gradients, and galvanotaxis. I. Biol. Bull., vol. 43, no. 5.

Meigs, Edward B. 1909 Concerning the supposed connection between protein coagulation and the heat shortening of animal tissues. Am. J. Physiol., vol. 24, no. 1.

1910 The effects of distilled water and of various solutions on the weight and length of striated muscle. Am. J. Physiol., vol. 26, no. 11.

MOORE, A. 1902 On the effect of solutions of various electrolytes upon rigor mortis and heat rigor. Am. Jour. Physiol., vol. 7, no. 1.

TAYLOR, FLETCHER B., AND ALVAREZ, WALTER C. 1917 The effect of temperature on the rhythm of excised segments from different parts of the intestine. Am. J. Physiol., vol. 44, no. 3.

VERNON, H. M. 1899 Heat rigor in cold-blooded animals. Jour. Physiol., vol. 24, nos. 3 and 4.

REPLY TO THE REMARKS OF PROF. WILHELM MICHAELSEN CONCERNING THE PERICHAETA MEGASCOLIDIOIDES, GOTO AND HATAI, AND FURTHER OBSERVATIONS MADE ON THIS SPECIES ON THE RELATION OF BODY LENGTH TO THE NUMBER OF SEGMENTS AND OF SETAE

SHINKISHI HATAI

ONE PLATE AND TWO TEXT FIGURES

Since Perichaeta megascolidioides is the name given to a single alcoholic specimen which was kept in the Zoölogical Department of the Tokyo Imperial University, consequently many other interesting features of this species were unknown at that time (1898). Recently I was very fortunate in being able to collect this earthworm in large numbers and was thus enabled not only to verify our former studies ('98), but also was given an opportunity to observe their behavior in their natural habitat. I propose, therefore, in the following pages, 1) to present the data on some anatomical characters, since Professor Michaelsen ('00) questioned the correctness of our observations, and, 2) to present some observations made on the interesting relations which exist between the body length and the number of segments as well as of setae. For convenience, I shall quote from our study the chief external characteristics of this species. "A large specimen 240 mm. long and 15 mm. wide in the thickest portion; number of segments 118. Number of setae in the spermathecal region 48. Clitellum very prominent, xiv-xvi, brownish in color, with a chaetal line to each segment, although there are no setae. Spermathecal pores 5 pairs, in iv/v-viii/ix, externally invisible. Male pores in xix, separated by 13 setae, and about 6.5

mm. apart. One pair of genital papillae about 0.5 mm. in diameter in segments xvii, xviii, xx, close to the posterior borders of the segments. First dorsal pore in xii/xiii."

The Perichaeta megascolidioides is the largest earthworm which I have been able so far to find in Japan and is quite common in both fields and open plains in the neighborhood of the city of Sendai and is often found even on little-used streets of the city after a heavy rain. The worm measures almost 1½ feet when fully extended and its unusually slow movement adds gracefulness to its size.

When, however, picked up suddenly or when disturbed by any means the animal contracts both ends of the body strongly and remains motionless. In this respect the megascolidioides behaves totally different from all other perichaetes so far as I have observed. When fully contracted the body measures about one-third of its fully extended length. The contracted worm then resembles a baseball bat having, however, two enlarged ends. This peculiar behavior is shown even in very immature specimens whose bodies measure only some two or three inches even when fully stretched out, and it enables us to identify this species at all stages of life, mature or immature, and thus it serves as one of the most desirable materials for various researches.

Perichaeta megascolidioides thus possesses several characters which serve to distinguish this species from other perichaetes; nevertheless, the openings of the male ducts in the nineteenth segment place this worm as one of the most unique species belonging to the genus Perichaetidae. It was this peculiarity which led Michaelsen to question the correctness of our observations and to make the following remarks:

"Gürtel borstenlos, aber mit sichtbaren Borstenzonen, vom 14-16 Segm. (=3). [Vermeintliche Borstenzone des 15 Segm. ist Intersegmtf. 14/15? Gürtel vom 14-15 Segm. (=2)]. Männliche Poren 5 mm. von einander entfernt, 13 Borsten zwischen sich fassend, am 19 Segm. (Misbildung?, oder am 18 Segm.?). 1 Paar Pubertätspapillen hinten am 17, 18 und 20 (16, 17 und 19?)."

Recently I have collected more than 150 individuals of various sizes belonging to this species and made careful studies on eighty well-preserved specimens and on more than fifty living worms, some of which are still kept in the laboratory for the purpose of various physiological investigations. The results of the reexamination show that without a single exception the male ducts open externally in the nineteenth segment as formerly found and not in the eighteenth segment as Michaelsen suspects. So far as the genital papillae are concerned there are some variations in regard to their positions as well as their numbers, but the degree of variability is astonishingly small when compared with those met with in some other species of Perichaetes as, for instance, in the Perichaeta hilgendorfi, Michaelsen. Among eighty preserved specimens I have found twelve individuals which showed either an excess or deficiency of one or more papillae, but in no instances were the male ducts noted opening in other segments than in the nineteenth.

The external openings of the male ducts are clearly visible long before the clitellum becomes visible, and thus in such individuals one can count the exact number of the segments without any fear of possible indistinctness of the segmental markings in this clitellar region in the adult specimen. In some individuals the clitellum is fairly well developed and the epidermis much swollen, exhibiting already the typical brownish color; nevertheless the intersegmental lines are still as distinctly present as in much younger specimens in which the clitellar region is indistinguishable from the rest of the body. In such individuals as just mentioned, we find most clearly that the clitellum consists of three segments, fourteenth, fifteenth, and sixteenth, and not of two segments, fourteenth and fifteenth, as suspected by Michaelsen. I may say that in the Perichaeta the composition of the clitellum of two segments instead of the usual three would be just as extraordinary as the male ducts opening in the nineteenth segment. Why, then, Michaelsen is more desirous to discover a perichaetous worm whose clitellum is short of one segment than a species having the male openings in the nineteenth instead of the eighteenth segment, is difficult to understand.

I can present one more evidence that the clitellum is made up of three segments and not of two segments, from the fact that the setae which are found on the fourteenth, fifteenth, and sixteenth segments, in fairly well-grown specimens, are definitely smaller than those found on the rest of the segments and remain smaller until these drop off as the epidermal tissue becomes modified to the typical clitellum. The fact just stated also shows that the clitellum is composed of the three segments as was found earlier.

I, therefore, conclude that Perichaeta megascolidioides possesses the external openings of the male ducts in the nineteenth segment as we have formerly found and the observations made on more than 100 specimens show decisively that this character is neither 'Misbildung' nor 'miscalculation' of the segments as was suspected by Michaelsen.

The oviduct opens, as our previous observations show, on the fourteenth segments and I have not found even one instance of exception. I may say that since this species possesses all other characters conforming in every respect with those of the genus Perichaetidae it would hardly be justifiable to group the present species as a new genus from the fact that a single aberrant specimen has the male openings in the nineteenth instead of the eighteenth segment.

Beddard ('95) called attention to the peculiarity of the Japanese Perichaetes in which are shown the tendency of both the male openings as well as of the spermiducal glands to disappear. I have recently collected several thousands of earthworms belonging to this genus and was surprised to find a disproportionately greater number of cases in which the external openings of the male ducts are totally absent. Furthermore, a considerably greater number of worms possess one unpaired opening in the eighteenth, or one in the eighteenth and another either in the seventeenth or in the nineteenth, or even in between the segments xvii/xviii or xviii/xix. These peculiarities of the Japanese Perichaetes, as had been noted by Beddard, together with a high degree of variability in the openings of the male ducts, which was noted by the present writer, would justify us in concluding that the position of the external male openings is not so stable a specific character, and thus Michaelsen's preconceived notion that the genus Perichaetidae should (or must) possess the external openings of the male ducts in the eighteenth segment must be discarded. The present writer is now collecting extensive data on the variability of the several characteristics of earthworms and therefore will reserve further discussion of this question to the near future.

THE RELATION BETWEEN THE BODY LENGTH AND THE NUMBER OF THE SEGMENTS

While collecting a large number of the earthworms with the intention ultimately to study the distribution of these worms in Japan, the present writer attempted to find some earthworms which would answer best for the purpose of general physiological researches. For such investigations the following qualifications are important: 1) The animals must be easily obtainable; 2) the body must be large enough for macroscopical anatomy and most of the organs must be capable of being isolated without very great difficulty; 3) the body must be elastic enough to resist a certain degree of pulling; 4) the animal must possess some characteristic features for easy identification, and, finally, 5) the species must be capable of identification at all stages of life, mature or immature, by a simple inspection.

There are numerous species of earthworms which answer several of these qualifications mentioned above, but so far as I am aware none, perhaps with the exception of the two species, Perichaeta megascolidioides, Goto and Hatai, and Perichaeta hupeiensis, Michaelsen, possess all those specified features. It is thus the intention of the present writer to carry on investigations as minutely as possible on the anatomical characters as well as the histological structures of these two species concerned for the purpose of future reference. As the first step I have studied the question concerning the constancy of the number of segments and of the setae in Perichaeta megascolidioides, and the results are presented in the following pages.

TABLE 1

The number of segments in the worms having varied body length

BODY LENGTH	NO. OF SEGMENTS	BODY LENGTH	NO. OF SEGMENTS	BODY LENGTH	NO. OF SEGMENTS	BODY LENGTH	NO. OF SEGMENTS
mm.		*mm.*		*mm.*		*mm.*	
77*	116	169*	114	198	116	223	116
79*	115	169	115	198	117	226	104
83*	117	171*	93	200	123	226	117
86*	109	173	117	200	118	229	118
92*	116	175*	116	200	116	230	116
95*	118	175	115	202	118	232	117
96*	117	175	117	206	116	234	115
103*	117	178	116	207	118	235	117
105*	117	179	116	208	117	239	116
111*	114	180	92	208	117	241	119
117*	115	181	106	211	103	244	117
132*	113	182	117	211	117	246	117
139*	117	183	115	213	119	248	117
148*	117	184	116	215	117	248	117
150*	114	185	103	216	100	250	117
150*	115	187	117	216	116	250	117
153*	117	188	116	219	116	251	119
167	116	188	116	220	117	258	117
168*	117	191	117	220	116	258	117
168*	116	192	117	220	116	259	116

*Immature: the clitellum not developed.

For this purpose eighty individuals altogether, with body lengths ranging from 77 mm. to 259 mm., were employed. The number of segments corresponding to the body length is shown in table 1.

In order to show the relation between the body length and the number of the segments more clearly, I have prepared for correlation table 2, based on the data given in table 1.

From table 2 we find that the number of segments which occur most frequently lies between 116 and 118, as shown in fifty-two cases out of eighty, or as much as 65 per cent of the total. The cases having the number of segments between 114-116 or 118-120 follow in the order mentioned. The individuals with the number of segments less than 114 are very few, but are scattered along a wide range of body lengths. It is a noteworthy fact that the cases having as small a number of segments as 92 to 114 are found among larger adult worms rather than among the small immature individuals. This fact indicates at once that the smaller number of seg-

TABLE 2

The correlation between the body length and the number of the segments

BODY LENGTH, MM.	NUMBER OF SEGMENTS																	MEAN NUMBER OF SEGMENTS
	92-94	94-96	96-98	98-100	100-102	102-104	104-106	106-108	108-110	110-112	112-114	114-116	116-118	118-120	120-122	122-124		
70-80												1	1				2	116
80-90									1				1				2	113
90-100													2	1			3	118
100-110													2				2	117
110-120												2					2	115
120-130																	0	...
130-140											1		1				2	115
140-150													1				1	117
150-160												2	1				3	116
160-170												2	3				5	116
170-180	1											1	5				7	113
180-190	1					1		1				1	5				9	111
190-200													4				4	117
200-210													4	3		1	8	119
210-220					1	1							4	1			7	113
220-230							1						6	1			8	116
230-240												1	3				4	117
240-250													4	1			5	117
250-260													5	1			6	117
	2	0	0	0	1	2	1	1	1	0	1	10	52	8	0	1	80	116

ments is not due to the immaturity of the worms concerned, but can best be interpreted as individual peculiarities. Indeed, we actually find small immature worms possessing as many segments as the mature worms, whose bodies, however, measure three or even four times longer. I have shown in figure 1 a graphic representation of the frequency distribution of the number of the segments based on the correlation table.

In this figure we find the relations which were stated in the preceding paragraphs more clearly shown, and when we consider the disproportionate abundance of cases having the number of segments between 116 and 118, and particularly when we consider that within this highest frequency are involved worms nearly all of different sizes, we can make no other interpretation from the distribution of the frequencies but that the number of the segments is fixed at an early stage of life and consequently the number of the segments does not increase with the increase in body length.

I have computed the mean values of the number of the segments corresponding to the given body length and the results are shown in figure 2.

Despite the greater irregularities of the results on account of the small number of observations, it seems clear that there is not even a tendency in the number of segments to increase, associated with the increase of the body in length. We therefore arrive from the present results at the same conclusion as before, that the number of the segments is early fixed and that the growth of the earthworm is accomplished by a passive elongation and not by the addition of newly formed segments. How universal the present conclusion will be when extended to other species of earthworms would be an interesting point to determine.

From the mean values already given we find that the average of the number of the segments is 116 and therefore the number of segments given by the original specimen comes close to this average value. (It was found to be 118.)

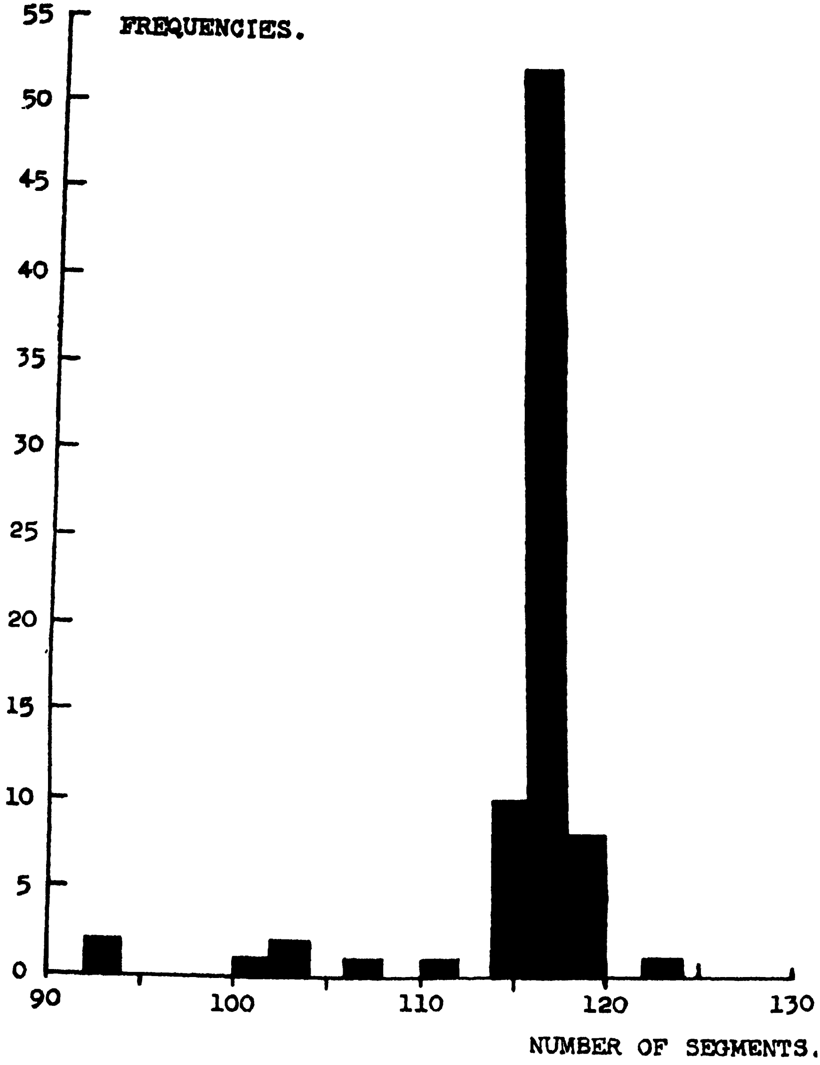


Fig. 1 Frequency distribution of the number of the segments.

One may question whether the fewer number of segments in some individuals may not have been caused either by the accidental loss of the posterior end or by an imperfect regeneration of the lost segments. It is, of course, conceivable that such might be one factor of the variabilities, but when one examines the external features of this species carefully such a conception becomes less probable for the following reasons.

The body of the worm exhibits a higher degree of symmetry and regularity than might be noticed by some casual observers. In the preserved specimens we notice that the width of the segments along the longitudinal axis of the body gradually narrows posteriorly, but at the same time the thickness of the body becomes greater from the middle part toward the posterior end. This thickness of the body reaches a maximum at the neighborhood of the anus or more precisely some fifteen or twenty segments in front of the anus. From this region caudally the thickness of the body steadily but not abruptly diminishes, tapering gradually toward the anus. Among preserved specimens I have noted several worms, however, which lack this sort of harmonious conformity, and the tail end possesses segments which in form are nearly the same as those taken from the middle part of the body without showing either the characteristic enlargement of the body toward and near the posterior end or the gradual narrowing of the rings. These individuals are certainly abnormal or most likely these are worms whose posterior part had been lost. These specimens were therefore discarded from the data presented here. But however short the length of the body or however few the number of segments, so long as the body of the worms showed symmetrical and harmonious conformity, as stated above, they were included among the normal specimens. It seems improbable that a worm whose organization is so complex and whose bodily features are so symmetrical and harmonious as the earthworm, can ever reduce its former regularity to an unrecognizable extent by a simple regeneration. So far as my rather limited experience goes, even with the Allolobophora, whose body is well known as possessing the wonderful power of regeneration, it is not a difficult matter to recognize the point at which either the new tail or the head end was reproduced.

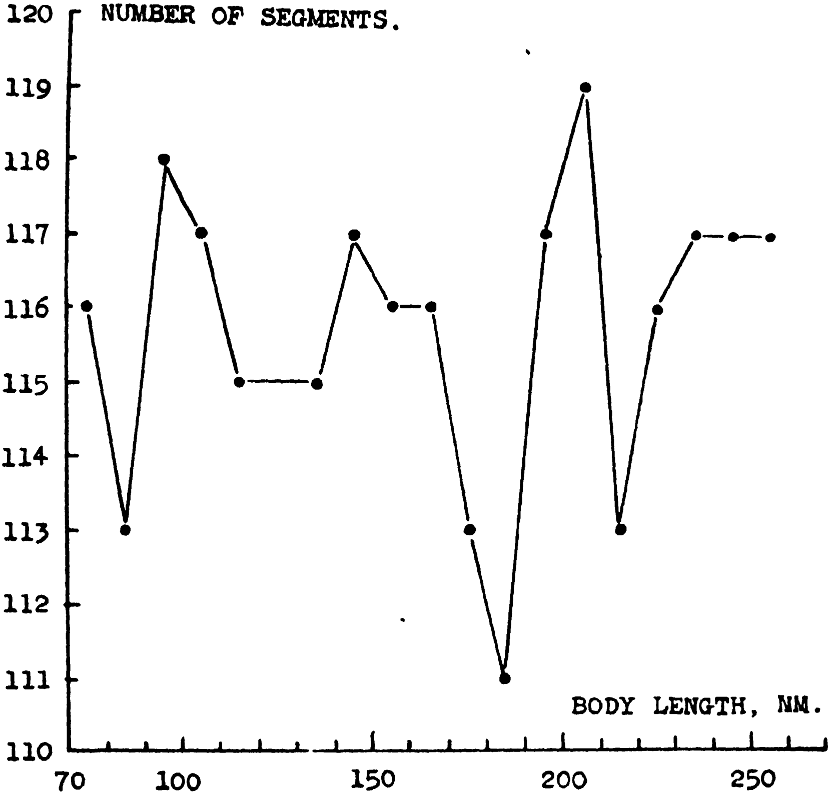


Fig. 2 The relation between the body length and the number of the segments.

THE NUMBER OF SETAE ON EACH SEGMENT

The number of setae which are found on the surface of the body were counted by numerous investigators, and were assumed by some, as it is one of the important characteristics for differentiation of the species. Most investigators, however, were contented to count the number of setae in a few segments only; that is, those found in three or four anterior segments or in one or two segments in the middle of the body. The segments chosen for this purpose were not the same but each investigator selected them according to his own convenience. Bourne (Beddard, '95) used the segments v, ix, and xxv (the latter segments serving as a type for the rest of the body), while Beddard ('95) selected segments ii, vi, xii, and xxvi. Beddard counted the number of setae on the anterior segments of the six different species of Perichaetes and demonstrated the wider differences according to the species concerned in the number of setae in corresponding segments.

So far as I am aware, no investigators ever took the trouble to count the total number of setae in all the segments throughout the entire length of the body, and we do not yet know with certainty the numerical relations of setae in each segment. Bourne's assumption that segment xxv is a type for the rest of the body can only be accepted after such enumeration on all the segments has been made at least upon several species. This tedious investigation has been carried out by the present writer with the additional intention of obtaining data as to the question of an early completeness of other anatomical characters.

I have counted the number of setae on five individuals, whose body lengths after preservation in formalin measure 77, 79, 168, 230, and 250 mm., respectively, so the samples here chosen will represent practically the smallest which can be found ordinarily and also the largest specimens which are usually obtainable. The results of the counts are given in table 3.

In order to show the relations more clearly I have plotted the number of setae on the corresponding segments and the results are shown in figure 3. The curves are badly crowded, but I believe the extent of variability, the increase in the number of setae toward the middle of the body and their decrease toward the anal segments, and high individual variations are better brought out in this chart than by merely glancing at the table. From both the table and the chart we notice the following points:

1. The number of setae increases steadily from the second segment up to about the twenty-fifth segment, from which it remains generally constant up to about the hundredth segment. Posterior to this region just stated, the number of setae begins to diminish very steadily and is totally absent in the remaining last few segments.

2. The number of setae on the corresponding segments differs according to the individuals, but the differences are not so large as might be anticipated. The number of setae varies within the individual from segment to segment, yet when the averages which were taken from several consecutive segments are compared with each other the results are closely similar, with of course exceptions of the anterior as well as posterior segments in which region the number of setae is very small. It seems desirable, therefore, or is rather necessary to count the number of setae in several consecutive segments when one intends to compare in several species the character concerned.

3. The setae on the clitellar segments are present in the younger and immature worms, but are entirely absent in the adults.

4. The setae which are found in the anterior preclitellar segments and those in the posterior anal region are larger and longer than those in the rest of the segments. Furthermore, the setae on the ventral surface appear to be slightly stouter than those found on the dorsal surface.

The total number of setae which are found in each individual are closely similar, as will be seen from the following relation:

Body length	77 mm.	79 mm.	168 mm.	230 mm.	250 mm.
Total number of setae...	8280	8536	8355	8632	8359

The above values were obtained by subtracting for the younger specimens the number of setae which are found in xiv, xv, and xvi segments (clitellum); that is 202 setae from 77 mm., 220 setae from 79 mm., and 206 setae from 168 mm. I have also added 836 setae in the place of the ten missing segments in the worm 168 mm. long. These values were

TABLE 3

The number of setae in each segment in five individuals having widely different body lengths

NUMERICAL ORDER OF SEGMENTS	77 MM.	79 MM.	168 MM.	230 MM.	250 MM.	NUMERICAL ORDER OF SEGMENTS	77 MM.	79 MM.	168 MM.	230 MM.	250 MM.	NUMERICAL ORDER OF SEGMENTS	77 MM.	79 MM.	168 MM.	230 MM.	250 MM.
1	—	—	—	—	—	41	79	86	84	93	86	81	76	80	82	74	80
2	28	31	33	30	27	42	83	88	82	90	88	82	81	82	83	76	75
3	40	38	39	41	38	43	82	85	80	97	84	83	79	83	83	76	77
4	43	44	44	45	44	44	84	93	85	96	89	84	83	79	82	76	80
5	49	46	49	58	51	45	77	91	83	94	85	85	77	80	80	77	80
6	53	49	55	55	53	46	78	87	82	93	89	86	78	81	82	75	78
7	54	50	54	56	58	47	80	88	83	91	88	87	78	82	79	77	77
8	58	56	56	60	62	48	83	94	90	92	89	88	82	84	80	78	77
9	59	57	56	62	62	49	82	92	86	91	84	89	80	85	84	77	79
10	59	64	62	60	61	50	76	95	87	88	91	90	81	78	83	75	77
11	59	64	61	61	64	51	80	88	85	86	84	91	81	84	83	78	76
12	60	62	64	60	62	52	80	90	85	91	86	92	79	83	80	80	76
13	65	70	64	62	63	53	78	91	85	91	91	93	80	79	82	79	82
14	63	72	66	—	—	54	79	86	86	91	85	94	80	84	79	78	77
15	65	72	69	—	—	55	80	90	86	87	88	95	80	82	81	79	79
16	74	76	71	—	—	56	82	86	84	89	86	96	76	79	80	79	80
17	71	76	67	76	69	57	82	88	88	88	85	97	78	84	83	81	83
18	72	76	73	69	69	58	82	82	88	87	86	98	76	82	80	82	81
19	69	78	61	64	63	59	83	84	85	84	84	99	76	77	83	82	76
20	72	77	72	74	75	60	80	83	81	88	85	100	78	78	74	87	81

21	73	80	72	81	78	61	81	85	83	88	86	101	78	80	77	79	81
22	78	80	81	84	81	62	76	90	85	88	87	102	80	79	75	82	76
23	78	83	76	87	79	63	77	88	87	86	84	103	77	75	80	78	73
24	80	80	77	85	84	64	77	86	84	82	87	104	79	80	76	78	71
25	83	79	79	79	82	65	76	88	83	84	85	105	79	76	75	78	71
26	84	88	81	89	80	66	77	89	—	88	84	106	75	74	72	74	66
27	80	84	82	84	80	67	75	84	—	88	80	107	76	75	67	70	69
28	76	88	80	94	83	68	83	82	—	89	78	108	73	70	71	71	66
29	81	86	83	90	84	69	76	78	—	85	83	109	71	61	61	65	60
30	82	86	79	90	84	70	76	89	—	85	84	110	63	44	60	56	50
31	80	90	80	91	85	71	81	88	—	82	82	111	61	30	56	63	49
32	80	92	83	95	86	72	77	86	—	76	84	112	56	8	47	63	40
33	84	87	84	88	88	73	76	88	—	77	86	113	51	—	19	54	16
34	76	93	81	88	87	74	76	83	—	81	80	114	48	—	—	—	—
35	80	91	83	94	89	75	75	88	—	80	83	115	40	—	—	—	—
36	80	87	82	95	84	76	78	85	79	80	82	116	25	—	—	—	—
37	85	88	84	92	83	77	76	89	86	79	83	117	—	—	—	—	—
38	79	90	85	90	84	78	79	86	85	75	83	118	—	—	—	—	—
39	81	86	81	90	90	79	81	83	85	79	80	—	—	—	—	—	—
40	78	89	83	90	87	80	77	85	82	78	76	—	—	—	—	—	—

found by adding ten segments to the average number between the anterior as well as the posterior five segments counting from the missing region. The final averages of the five individuals give 8432 setae or approximately 8500 setae per individual.

We find from the above that so far as the present observations are concerned there is no indication of increase in the number of setae associated with the growth of body. Although the smallest individual (77 mm.) happened to give the least number of setae (8280) at the same time another individual measuring but 79 mm. gives a far greater number of setae (8536) than the largest worm (250 mm.) which gives 8359. If we arrange these worms according to the numerical superiority of the setae we obtain the following relation:

Body length	77 mm.	168 mm.	250 mm.	79 mm.	230 mm.
Total number of setae .	8280	8355	8359	8536	8632

From the above relations we will be justified in concluding that the number of setae in the Perichaeta megascolidioides is fixed at an early stage of life and no new additions are probably made during subsequent growth of the body. The variations here noted, therefore, can be considered as 'individual,' and thus within this narrow limit we may conclude that the number of setae is constant.

We have, then, in this species of the earthworm at least, two anatomical characters, the number of segments and the number of setae, which show early completion, and there is a high degree of probability that these characters are constant within the narrow range of individual variation. This fact just found will materially simplify the interpretation whenever the problems are concerned with the segments or with the setae of this species.

LITERATURE CITED

BEDDARD, F. H. 1895 A monograph of the Order of Oligochaeta. Oxford. Clarendon Press.

GOTO, S., AND HATAI, S. 1899–1901 New or imperfectly known species of earthworms. No. 2. Annotationes Zoologicae Japonensis, vol. 3.

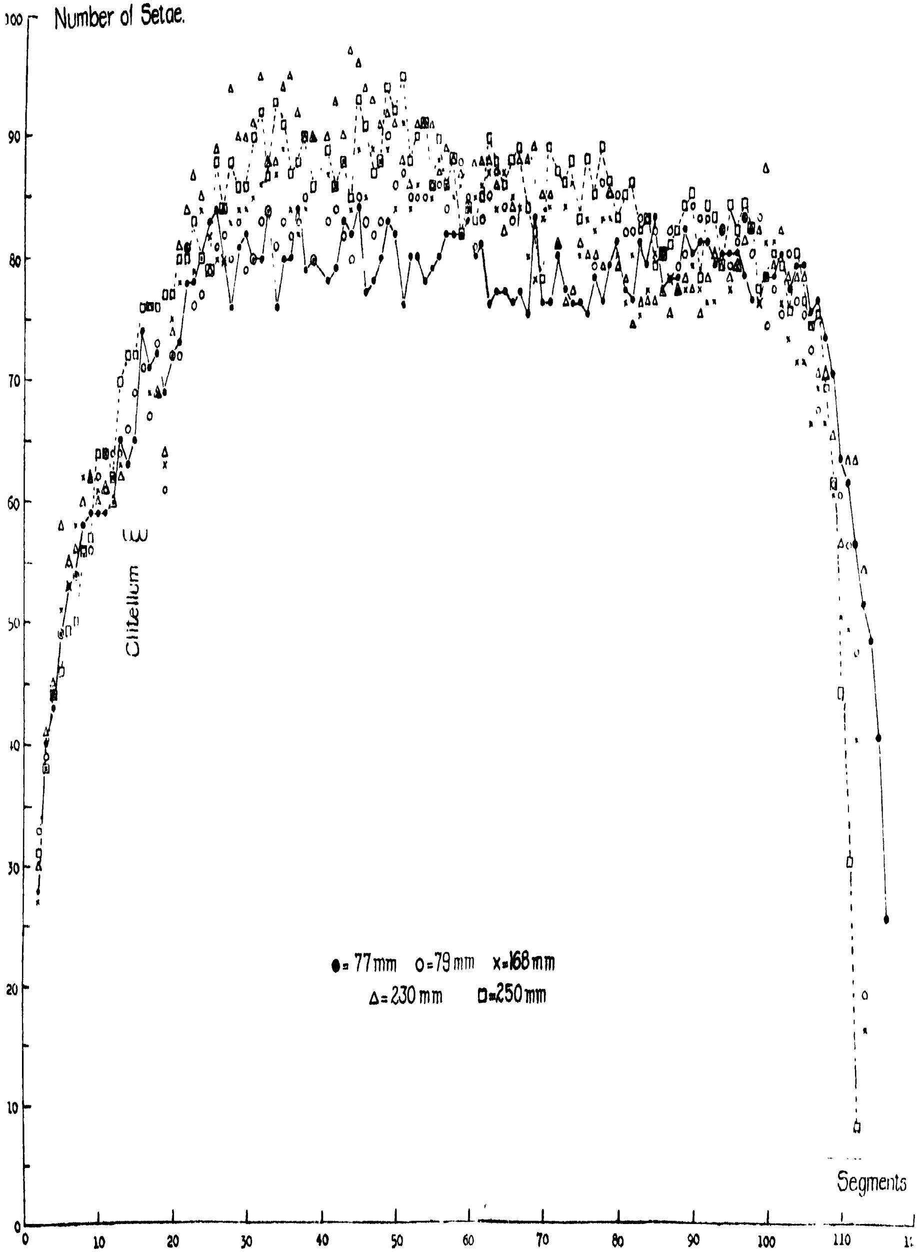
MICHAELSEN, W. 1900 Oligochaeta. Berlin. Friedländer und Sohn.

PLATE

PLATE 1

EXPLANATION OF FIGURE

3 The number of setae on each segment throughout the entire length of the body of Perichaeta megascolidioides, Goto and Hatai.


Number of Setae.
100
90
80
70
60
50
40
30
20
10
0
Clitellum
●= 77 mm ○= 79 mm ×= 168 mm
△= 230 mm □= 250 mm
Segments
0
10
20
30
40
50
60
70
80
90
100
110

DE NOVA ASARI SPECIE EX JAPONIA AUSTRALI

TOKUTARO ITO

CUM TABULA 1

ASARUM FUDSINOI (T. Ito, species nova)

Species A. maximo Hemsl. proxima, differt calyce intus reticulato non transversim multiplicato; etiam A. macrantho Hook. fil., similis, recedit lobis perianthii non valde undulatis, tubo intus non multiplicato et positioni stigmatis.

Herba robusta, fere undique glabra, perennis, rhizomate repente, fibris radicalibus crassis; internodiis abbreviatis. Folia 1 vel 2–3, ampla, longissime petiolata, ovato-cordata vel cordata, ad apicem attenuata, absque petiolo fere 20 cm. longa, 13 cm. lata, subcoriacea, obtusa vel subacuta, integra vel leviter subundulata, lobis latis rotundatis, saepe imbricatis, venis primariis 5 vel 7 crassis; folia pauca secondaria minora, lanceolato-vel ovato-cordata vel cordata obtusa. Petiolus 30 cm. longus, crassus, subcarnosus, fere teres. Flores 1–3, subdeclinati, "extus luteoviridescentes, intus rubro-purpurei" (e Fudsino in litt.), 3½–5 cm. diametro, breviter pedunculati, pedunculo bracteato, bracteis 3, ovatis, subacutis, 1½–2½ cm. longis; perianthium subaequaliter trilobatum, lobis fere 1½ cm. longis latisque, late ovatis vel subdeltoideis obtusis planis divergentibus, tubo intus elevato-reticulato; antherae subsessiles, uniformes, oblongae, minute appendiculatae, appendiculum membranaceum obtusum. Ovarium latum, basi subsessile. Styli stamina superantes, crassi, biolobati, stigmate ovato dorsali.

Japonice: Fudsino Kan-awoi (mihi)

Hab. in Japonia australi: in vallibus montanis prope portum Naze, insulae Amami-Oshima, cl. K. Fudsino (3 Novembris, 1906, steril. et 5 Januarii, 1907, florens) legit et adumbrationem plantae florentis cum figuris analyticis misit.

TABULA

EXPLICATIO TABULA 1

Asarum Fudsinoi T. Ito

1 Habitus. Magn. nat.

2 Flos cum perianthio longitudinaliter fisso et androecium gymnoeciumque exhibentur.

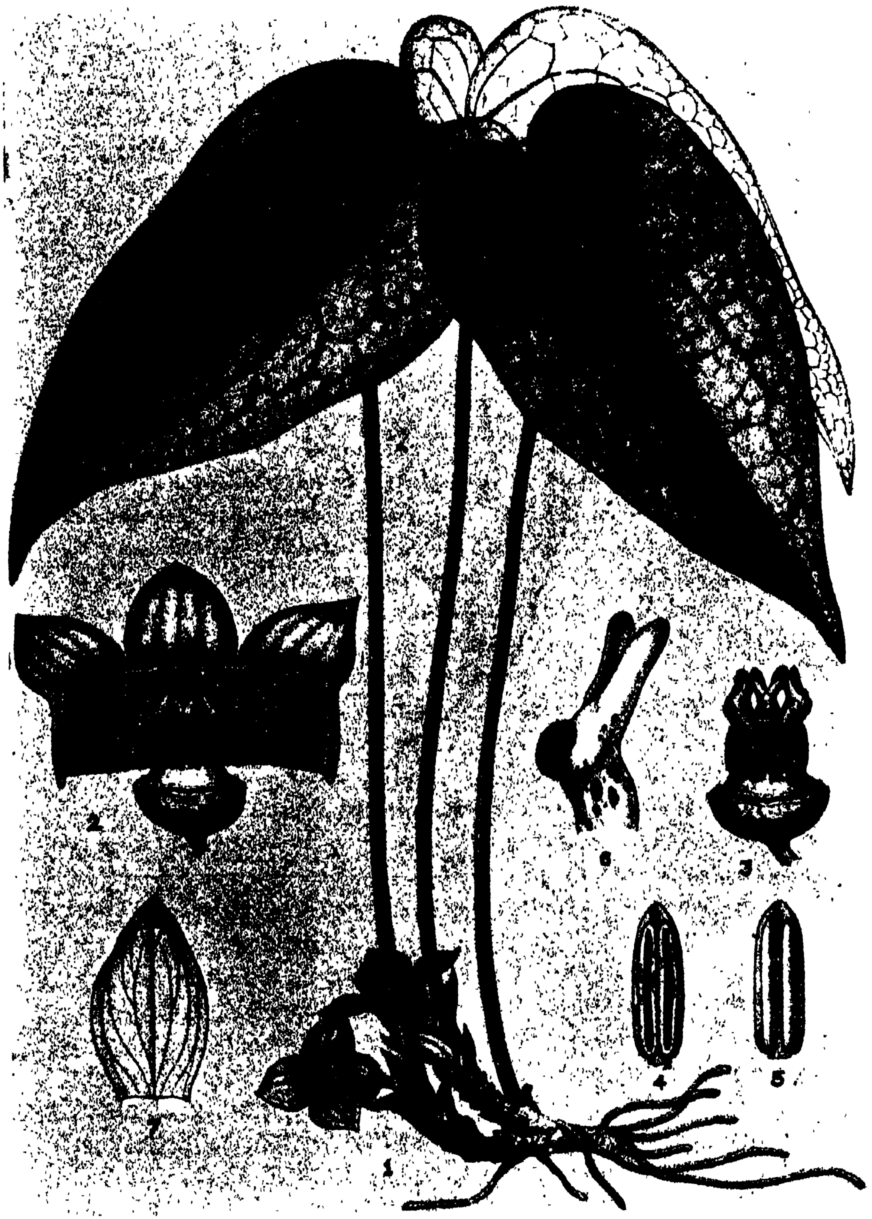
3 Androecium et gymnoecium.

4 Anthera a ventre.

5 Eadem a dorso.

6 Stylus et stigma.

7 Bractea.



Sakuma, ad sicc. del.

METABOLIC ACTIVITIES OF THE NERVOUS SYSTEM

V. 3. ON THE REGULAR SEASONAL CHANGES IN THE RELATIVE WEIGHT AND THE SEX DIFFERENCE OF THE CENTRAL NERVOUS SYSTEM OF RANA NIGROMACULATA

SHIGEYUKI KOMINE

INTRODUCTION

The potential growth energy of each organism is characteristically the same for a given species. The human brain attains about 1350 grams, while that of the frog reaches 0.2 gram. The potential growth energy of the human brain is, therefore, 1350 grams and that of the frog 0.2 gram.

For the sake of convenience, this potential energy is called 'growth energy.' The growth energy inborn with the germ cell of an animal takes in the nutriments to construct its own body and to attain its maximum development. The maximum development of a grown animal is, therefore, to be regarded as an index for the measurement of the said energy. In the actual life, however, a part of the nutriment is transformed to kinetic energy and the rest to the material substratum deposited in the cell body to form gradually and progressively the mature organism.

For the thorough understanding of the above process the study of cell elements, chemical contituents, and the weights of the various organs in successive stages of development is of vital importance. It is for this reason that a number of valuable investigations have been carried out on this line, particularly by Donaldson (The nervous system of the American leopard frog, Rana pipiens, compared with that of European frogs, Rana esculenta and Rana temporaria ('08); On a formula for determining the weight of the central nervous system of the frog from the weight and length of its entire body ('02); On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog ('11)), and by Donaldson and Shoemaker (Observations on the weight and length of the central nervous system, and of the legs in frogs of different sizes ('00)) and by Snell (Die Abhängigkeit des Hirngewichtes von dem Körpergewicht und den geistigen Fähigkeiten ('92)), and by Dubois (Die Abhängigkeit des Hirngewichtes von der Körpergrösse bei den Säugethieren ('92)). Following the plan and methods of Donaldson's work, I have attempted to carry out a similar study in the hope of finding the difference in the central nervous system under varied conditions, such as locality, food, and climate.

An attempt has also been made to determine the sex difference in the weight of the central nervous system.

EXPLANATION OF THE FORMULA FOR THE DETERMINATION OF THE WEIGHT OF THE CENTRAL NERVOUS SYSTEM OF THE FROG FROM THE WEIGHT AND LENGTH OF ITS ENTIRE BODY

As I have adopted Donaldson's formula for determining the weight of the central nervous system of the frog from the weight and length of its entire body, it will be found convenient to give an explanation of it before discussing my own work. Donaldson is the first to figure out the formula showing the relationship between the weight of the central nervous system and the combined total length and the body weight. It seemed to him that the weights of the central nervous system arranged according to the body weight were so related as to suggest a logarithmic curve. Donaldson tried to form a curve depending on the logarithms of the body weights. To raise these logarithms to the value of the observed weights of the central nervous system they must be multiplied by a constant factor. It was found that the factor which gave a correct value for the smallest frog was too small for all of the succeeding cases, the resulting numbers falling more and more below the observed numbers as the frog became larger. In order to make the curve based on the calculated weights fit with that based on the weights observed, a second factor was needed, the value of which should steadily increase with the body weight of the frog, which increases rather rapidly at first and more slowly later. The unmodified lengths showed, however, too rapid an increase in the course of the series, but various trials revealed that the fourth root of the lengths gave a set of numbers which could be satisfactorily used. It was found that the number obtained by multiplying the logarithm of the body weight by the fourth root of the length of the body was always nearly a constant fraction of the observed weight of the central nervous system.

In the case of the bullfrog the fraction thus obtained was one-thirtieth of the observed weight, while in the case of the leopard frog it was one-twenty-eighth. It could, therefore, be made equal to the observed weight by multiplying it by a constant factor having the value of the denominator of the fraction. Thus the formula became as follows:

$$\text{Weight C.N.S.} = (\text{Log } W \times \sqrt[4]{L})\, C.$$

In the above formula the weight of 'C.N.S.,' central nervous system, is calculated in milligrams; 'W,' the weight of the body, is in grams; 'L,' the total length of the body, is in millimeters, and 'C,' the constant, is the sum necessary to raise the product of these factors to the observed weight.

TECHNIQUE

The material used was the Japanese frog, Rana nigromaculata, and the technique throughout the course of the research was essentially uniform. Frogs were obtained from brooks and rivulets which run into the Arakawa River, in Tokyo-fu. These were transported to a little pond 72 sq.ft. in area belonging to the laboratory and supplied with the weeds and insects. After the frogs were adapted to the new surroundings they were subjected to successive investigations. Previous to winter, in which the water was apt to freeze, they were caught and buried under ground with earth from rice fields, and were kept in the same condition as in their natural life.

The examinations were performed regularly in the latter part of the month in order to obtain uniform results. The frogs were kept moist for several hours before dissection. They were killed with ether and the body weight (Bd.W.) taken to the nearest 0.1 gram. The frog was next either suspended or laid flat on its abdomen, with the legs fully extended, and the distance from the tip of the nose to the tip of the longest toe taken with jointed calipers and then computed to the nearest millimeter (total length). While in the ventral position the long axis of the head was brought in line with that of the body by raising the head with a small wooden wedge, and the distance from the tip of the nose to the tip of urostyle—the cartilaginous end of which was exposed by a slit through the skin—was measured and read to the nearest 0.1 mm. (body length).

The frog was then placed on its back, opened, and all the viscera were removed. At this time any necessary correction in body weight was made by subtracting from the initial weight the weight of the ova or of the undigested food distending the stomach. These were the only corrections made to the body weight. The body weights of females are always given without the ova. Then the brain was exposed through its entire length and the spinal cord exposed as far down as the third nerve. With spring compasses the length of the brain from the tip of the olfactory bulbs to a point midway between the tip of the calamus scriptorius and the level of the second nerve was taken and was recorded to the nearest 0.1 mm. (brain length). The olfactory nerves were next cut through with very fine scissors, and in the same manner a section was made at the level of the second nerve. The brain was carefully taken out without the choroid plexus and the attached nerve roots were severed as close to the brain as possible.

If the hypophysis was still attached to the brain, it was removed and the remaining mass at once placed in a closed weighing bottle and weighed to the nearest 0.1 mg. (brain weight).

The spinal cord was exposed through its entire length and the conus just caudad to the ninth nerve laid bare. With spring compasses the length from this point to the place at which the cord had been severed from the brain was measured and recorded to the nearest 0.1 mm. (cord length). The cord was then taken with fine forceps just below the conus and raised so that the nerves could be clipped close to the cord. The mass of cord thus deprived of nerves was placed in a closed weighing bottle and at once weighed to the nearest 0.1 mg. (cord weight).

The central nervous system was then dried at 90 to 95°C. for a week and reweighed.

Data observed and calculated according to Donaldson's formula

As is shown in table 1, the observed and the calculated weights of the central nervous system are the same through four seasons of a year, both being 104. In Donaldson's investigation ('11) both the observed and calculated weights of the central nervous system of the leopard frog were 146, while the observed and calculated weights of the bullfrog showed a slight deviation, being 212 and 211, respectively. The leopard frog, when compared with the bullfrog, is less in weight, smaller in size, and relatively longer in body length in much the same proportion as Rana nigromaculata. This appears to be the reason why the observed and calculated weights of the central nervous system of Rana nigromaculata are identical with those of the leopard frog. Although a slight deviation is found between the observed and calculated weights of the central nervous system of the bullfrog, it represents only 0.4 per cent and should be regarded as almost insignificant. From what has been shown by Donaldson and from what we have obtained from the Japanese frog, Rana

TABLE 1

Containing eighty-eight records of Rana nigromaculata which are arranged according to ascending order of their body weights.. In this table is included the percentage deviation of the calculated from the observed weights of the central nervous system, the observed values being taken as the standard

TABULAR NUMBER	SEX M=Male F=Female	BODY		WEIGHT OF CENTRAL NERVOUS SYSTEM		PERCENTAGE DEVIATION OF CALCULATED FROM OBSERVED	
		Weight	Length	Observed	Calculated	Deficiency	Excess
		grams	*mm.*	*mg.*	*mg.*		
1	M	9.0	121	65	76	....	17.0
2	M	10.2	114	77	79	....	2.6
3	M	10.2	117	83	80	3.6	....
4	M	10.3	130	79	82	....	3.9
5	M	10.3	132	84	81	3.6	....
6	M	10.5	135	81	84	....	3.7
7	M	11.0	135	82	85	....	3.7
8	M	11.6	126	84	86	....	2.4
9	M	11.7	127	91	85	6.6	....
10	M	11.7	134	82	87	....	5.7
11	F	12.5	139	93	90	3.2	....
12	F	13.0	128	85	90	....	5.9
13	M	13.0	139	112	92	17.9	....
14	F	13.2	125	96	90	6.2	....
15	M	13.5	124	84	95	....	13.1
16	M	13.5	125	94	91	3.2	....
17	F	13.5	128	86	91	....	5.8
18	M	13.5	126	91	91	0	0
19	M	13.5	129	86	91	....	5.8
20	F	13.7	134	91	93	....	2.2
21	M	14.2	130	100	93	7.0	....
22	M	14.5	143	113	97	14.1	....
23	M	14.7	126	93	94	....	1.1
24	F	15.0	134	98	97	1.0	....
25	M	15.0	140	94	99	....	5.3
26	M	15.0	138	103	97	5.8	....
27	M	15.2	138	99	97	2.0	....
28	F	15.3	136	113	102	13.4	....
29	F	15.5	144	97	99	....	2.1
30	M	15.7	130	92	97	....	5.4
31	M	16.0	152	113	102	9.7	....
32	M	16.2	135	108	99	8.3	....
33	M	16.2	139	107	100	6.5	....
34	M	16.5	128	87	98	....	12.6
35	M	17.0	135	112	100	10.7	....
36	F	17.0	139	97	91	6.1	....
37	M	17.0	141	111	102	8.1	....
38	M	17.2	140	101	102	....	1.0
39	M	17.5	132	97	101	....	4.1
40	M	17.5	139	96	102	....	6.2
41	F	17.7	146	90	104	....	15.5
42	M	18.0	132	87	102	....	17.2
43	F	18.0	128	106	109	....	2.8

TABULAR NUMBER	SEX M=Male F=Female	BODY		WEIGHT OF CENTRAL NERVOUS SYSTEM		PERCENTAGE DEVIATION OF CALCULATED FROM OBSERVED	
		Weight	Length	Observed	Calculated	Deficiency	Excess
		grams	*mm.*	*mg.*	*mg.*		
44	F	18.0	134	95	103	....	8.4
45	F	18.0	139	93	103	....	10.7
46	M	18.0	142	117	104	11.1	....
47	F	18.3	139	96	104	....	8.3
48	M	18.2	132	110	103	6.4	....
49	F	18.3	139	95	104	....	9.5
50	F	18.5	147	110	106	3.6	....
51	F	18.6	136	103	104	....	1.0
52	F	18.7	136	105	104	1.0	....
53	M	18.7	140	105	106	....	1.0
54	M	19.0	142	122	106	13.1	....
55	F	19.0	144	117	106	9.4	....
56	M	19.0	145	110	107	2.7	....
57	M	19.5	143	99	107	....	8.1
58	F	19.5	144	118	108	8.5	....
59	M	19.5	145	113	107	5.3	....
60	M	20.5	152	118	111	5.9	....
61	F	20.5	154	135	111	17.8	....
62	F	20.5	144	101	110	....	8.9
63	F	20.5	146	120	109	9.2	....
64	F	21.0	144	101	110	....	8.9
65	F	21.0	148	97	113	....	16.5
66	M	21.5	151	117	112	4.3	....
67	F	21.5	168	129	115	10.9	....
68	F	22.0	143	112	111	0.9	....
69	F	22.0	145	112	112	0	0
70	F	22.0	145	112	112	0	0
71	F	24.5	154	111	118	....	6.3
72	M	24.7	145	116	116	0	0
73	F	25.0	135	106	114	....	7.2
74	F	25.2	151	110	118	....	7.5
75	F	25.2	159	122	119	2.5	....
76	F	25.8	149	107	115	....	7.3
77	F	26.2	158	136	121	11.0	...
78	F	26.5	153	101	116	....	19.8
79	F	26.5	157	110	121	....	5.5
80	F	26.7	160	101	122	....	20.8
81	F	28.0	158	122	123	....	0.8
82	F	29.2	165	116	126	....	8.6
83	F	30.5	165	137	128	6.8	....
84	M	31.5	160	122	128	....	4.9
85	F	32.0	168	135	130	3.7	....
86	F	35.0	166	123	133	....	8.3
87	F	44.0	172	142	143	....	0.7
88	F	47.7	185	172	150	13.8	....
				104	104	7.2	7.2

nigromaculata, it must be admitted that Donaldson's formula expresses most accurately the relationship existing between the weight of the central nervous system on the one hand and the body weight and the total length on the other. If the constant C is adequately modified, this formula is available

TABLE 2

Weight of the central nervous system in grams

	BODY WEIGHT	WEIGHT OF CENTRAL NERVOUS SYSTEM	TOTAL LENGTHS	OBSERVER	NUMBER OF FROGS
		gram	*mm.*		
R. pipiens	14.9	.1040	146	Donaldson	3
	23.2	.1256	171	Donaldson	3
	30.8	.1463	183	Donaldson	3
	43.2	.1674	198	Donaldson	3
R. esculenta	15.9	.0890	139	Donaldson	3
	22.6	.1142	164	Donaldson	3
	35.0	.1402	176	Donaldson	3
	40.2	.1498	193	Donaldson	3
R. temporaria	15.9	.0929	149	Donaldson	3
	23.1	.1108	167	Donaldson	3
	28.0	.1213	173	Donaldson	3
	31.3	.1323	176	Donaldson	3
R. nigromaculata	12.3	.0883	129	Komine	23
	17.4	.1032	139	Komine	36
	23.8	.1135	152	Komine	23
	32.0	.1292	165	Komine	4
	45.9	.1570	179	Komine	2

not only for American frogs, but also for Japanese as well as for European species.

In order to show the weight relations of the central nervous system found in the four different species of frogs I have made table 2, based on the records given in table 1, and have also included data on R. pipiens, R. esculenta, and R. temporaria, values which were determined by Donaldson ('01).

The relations which are shown in table 2 are better brought out in chart 1.

The curves run nearly parallel in spite of the difference of locality and species. It will be noted that the Rana pipiens of America is greater in the weight of the central nervous system

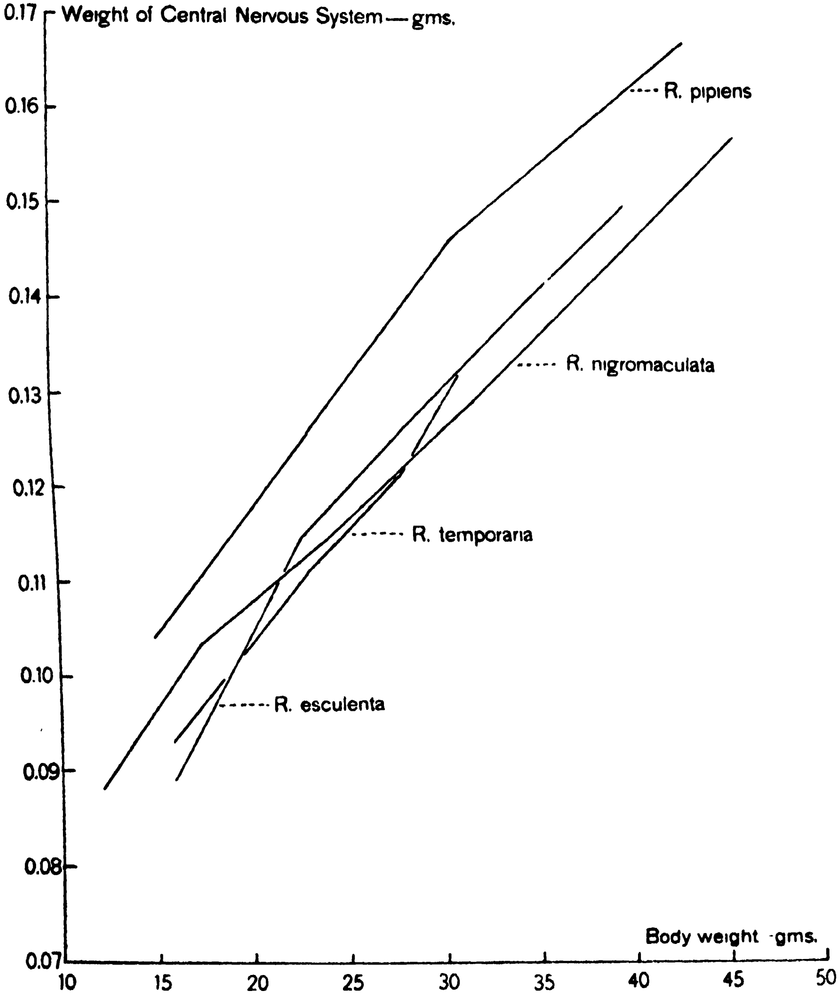


Chart 1 Showing the weight of the entire central nervous system of four different species of frogs on their respective body weights.

when compared with those found in the Rana nigromaculata of Japan, Rana esculenta and Rana temporaria of Europe, while those of the latter three species are very close to one another. The reader's attention is called also to the fact that the Rana nigromaculata shows a greater weight of the central nervous system in its early stage of development than do Rana esculenta and R. temporaria.

TABLE 3

Showing the value of the constant

SERIES	NUMBER OF SPECIMENS		DATE	VALUE OF C	
	M	F		MEAN	RANGE
1	5	4	October 21	23.4	(23.2–25.7)
2	3	3	November 30	22.9	(20.6–27.7)
3	2	2	December 24	23.5	(22.0–24.6)
4	2	3	January 27	23.5	(19.8–25.3)
5	3	2	February 22	22.8	(21.9–23.4)
6	3	2	March 29	23.8	(22.1–25.9)
7	3	2	April 20	23.3	(19.9–25.8)
8	2	3	May 24	24.2	(21.0–27.7)
9	3	3	June 20	24.5	(23.1–26.9)
10	3	3	July 25	26.0	(23.3–27.5)
11	4	5	July 31	25.0	(22.6–27.0)
12	5	3	August 25	25.2	(21.6–26.7)
13	2	2	August 31	24.0	(22.7–25.6)
14	3	8	September 20	24.0	(20.4–29.1)

Evidence of seasonal changes in the weight of the central nervous system

The value of the constant in each month is given in table 3. In table 4 are given the data on the body weight, total length, body length, the weight of the brain and cord, together with the data on the percentage of water in both brain and cord.

In order to get a sufficient insight into the relationship existing between the growth of the central nervous system and climate, the value of the various series (including Donaldson's investigations on frogs of Zürich and Liverpool, 1908, and on those of Chicago, Minnesota, and Brandywine in each succeeding month) is shown in table 5 and their graphs in chart 2.

From what can be seen in the chart, it is apparent that there exist rhythmic seasonal changes in the growth of the central nervous system of Rana nigromaculata as is the case in the other series. Roughly speaking, all series show the maximum relative weight of the central nervous system to be in the summer, the minimum in the winter, and intermediate in the spring and autumn.

TABLE 4

Giving the mean value of all the data for each month

NUMBER OF SPECIMENS		BODY WEIGHT	TOTAL LENGTH	BODY LENGTH	BRAIN			CORD		
M	F				Weight	Length	Per cent water	Weight	Length	Per cent water
		grams	*mm.*	*mm.*	*grams*	*mm*		*grams*	*mm*	
5	4	17.5	134.0	55.5	0.0685	13.2	84.1	0.0291	14.2	80.1
3	2	20.4	145.0	64.2	0.0707	13.4	84.5	0.0320	14.4	80.2
2	2	13.8	134.6	57.8	0.0589	12.7	83.8	0.0277	13.9	80.0
2	3	20.9	145.0	58.1	0.0702	13.4	84.3	0.0313	14.4	80.3
3	2	14.7	132.0	58.2	0.0626	13.1		0.0281	14.0	
3	2	17.9	142.0	60.6	0.0686	13.6	83.6	0.0320	14.6	79.8
3	2	18.4	134.0	59.6	0.0703	13.4	84.0	0.0304	14.5	80.4
2	3	19.8	141.0	62.6	0.0721	13.5	84.7	0.0313	14.5	80.4
3	3	18.7	143.0	62.1	0.0756	13.6	84.2	0.0327	14.6	79.9
7	8	19.6	144.0	59.8	0.0761	13.6	84.2	0.0356	14.6	80.4
7	5	19.3	143.0	59.3	0.0731	13.6	84.5	0.0351	14.6	80.8
3	8	21.2	146.0	62.7	0.0736	13.5	84.4	0.0354	14.6	80.5
41	44									

The highest value of the constant is found in the Minnesota series followed by the Chicago, Brandywine, and Japanese in order. The European series show the lowest value of the constant. The Brandywine series and the Japanese are the closest with regard to the value of the constant, only differing in that the former attains its maximum in May and the latter in July. The Minnesota and Chicago series show the maxi-

TABLE 5

The data on the value of constant in different species of frogs in different localities

	DONALDSON ('11)					Tokio R. nigromaculata 1922
	Chicago R. pipiens 1901–02	Minnesota R. pipiens 1908	Brandywine R. pipiens 1909	Zürich R. temporaria	Liverpool R. temporaria	
I. January	24.8 (I, 30)					23.5 (I, 27)
II. February			23.7; 23.8 (II, 4) (II, 22)			22.8 (II, 22)
III. March	24.7 (III, 28)	28.1 (III, 26)	24.0 (III, 23)			23.8 (III, 29)
IV. April	25.8 (IV, 9)		24.8 (IV, 21)			23.2 (IV, 20)
V. May	27.0 (V, 4)		26.8 (V, 20)			24.2 (V, 24)
VI. June	27.6; 25.8 (VI, 1) (VI, 29)	31.1 (VI, 10)	26.3 (VI, 19)			24.5 (VI, 20)
VII. July	28.5 (VII, 27)			23.8^{x}; 23.7^{x} (VII, 20–'04) (VII, 6–'09) 22.8° (VII, 1–'04)	22.8 (VII, 1–'04)	26.0; 25.0 (VII, 25) (VII, 31)
VIII. August	28.3 (VIII, 24)		26.1 (VIII, 13)	21.8° (VIII, 20–'04)	21.9 (VIII, 20–'09)	25.2; 24.0 (VIII, 25) (VIII, 31)
IX. September	27.9 (IX, 22)		27.2; 25.2 (IX, 8) (IX, 24)			23.9 (IX, 20)
X. October		28.2; 28.3 (X, 19) (X, 19)	24.5 (X, 19)			23.2 (X, 21)
XI. November						22.8 (XI, 30)
XII. December						23.5 (XII, 24)

^{x} R. esculenta.

mum value of the constant in June and July, being similar to the Japanese series in this respect. It will be seen from the above that even the same species show different modes of development according to the surrounding conditions.

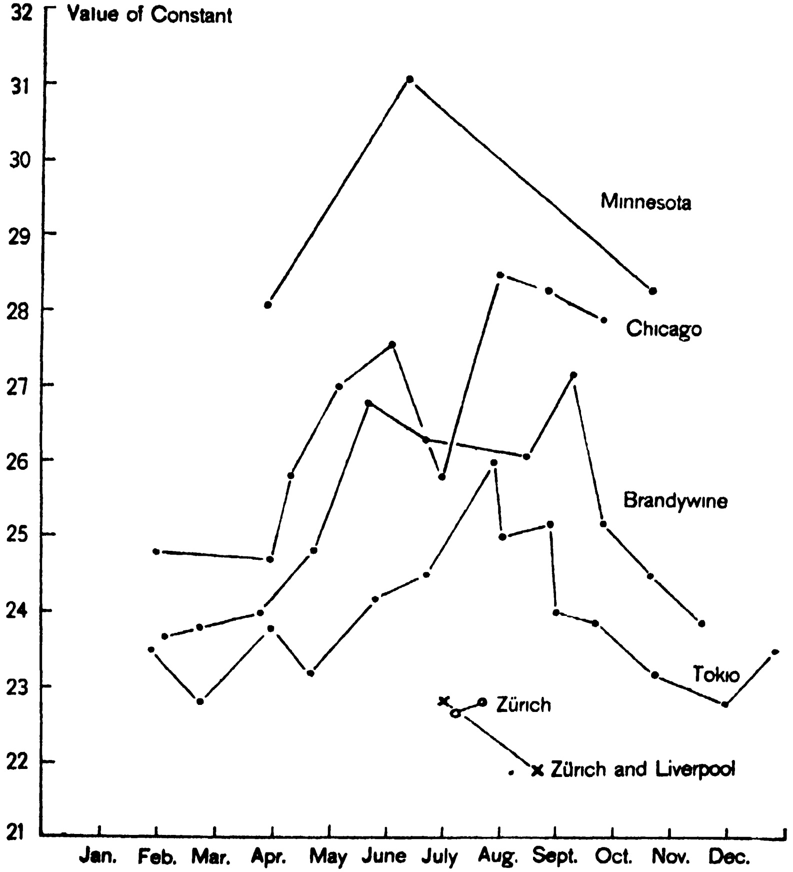


Chart 2 Showing the value of the constant of all the groups according to locality and season. R. esculenta X, R. temporaria O.

The fact that the American frogs show the highest value of the constant, the Japanese midway, and the European the lowest may partly be due to the difference of species, but it must be chiefly accounted for by the difference of climate, location, and food.

The values of the constant of the various series are tabulated as follows:

Chicago	24.7
Brandywine	24.0
Minnesota	28.1
Zürich	23.8
Liverpool	22.8
Japan	24.0

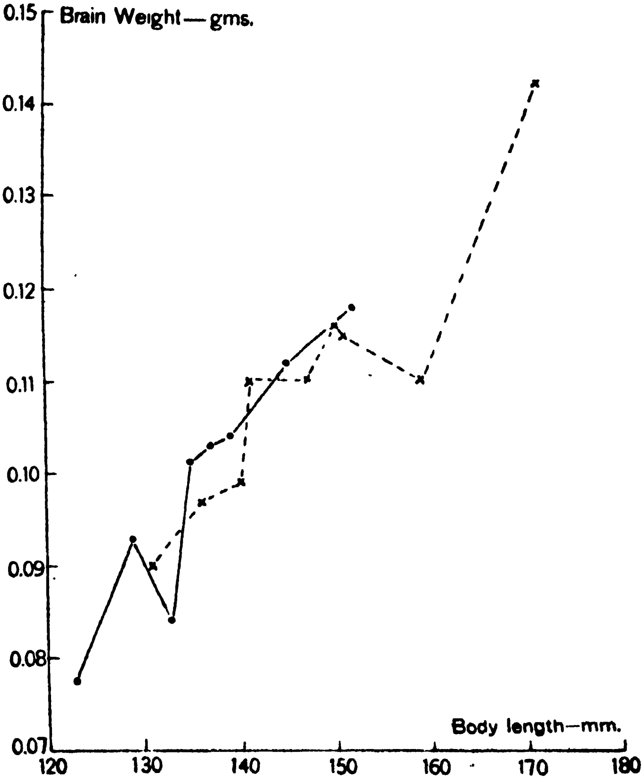


Chart 3 Showing the sex difference in the weight of the brain according to body length. Male, ————————. Female – – – – – –.

The values of the constant for each species of frogs are shown in the following table:

	C.
Rana pipiens	27.6
Rana esculenta	23.8
Rana temporaria	22.8
Rana nigromaculata	24.0

The difference in the weight of the central nervous system according to sex

In order to find out whether or not there is any difference in the weight of the brain between sexes I have plotted the weights of the brain for each according to the total length

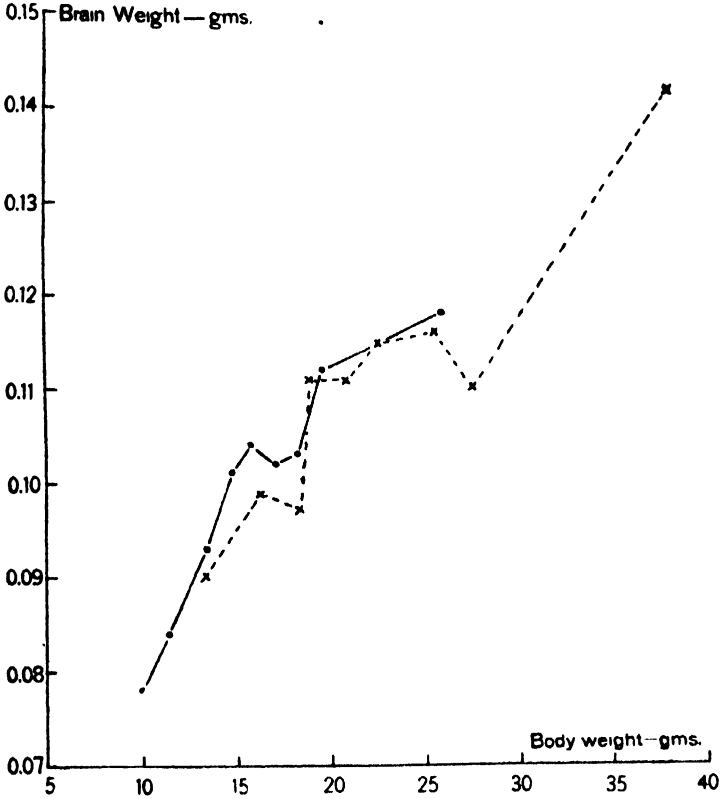


Chart 4 Showing the sex difference in the weight of the brain on body weight. Male, ——————. Female, – – – – – –.

and body weight in charts 3 and 4, respectively, based on the data given in table 6.

The evidence that there exists a sex difference in the weight of the brain has further been confirmed by the value of the constant arranged according to the month of a year. The facts just stated are well shown in chart 5 from values which are given in table 7.

It will be noted here that the curve of the constant for the male runs in general above that of the female, the average constant for the male being 24.4 and that for the female 23.7. The female constant for March is an exception from this general rule, since it is strikingly higher than that of the male. This fact seems to me to offer something very significant with regard to the sexual activities of the frog. To avoid experimental error, I have repeated the work for March a second time and have obtained the same result. Here it has been found that the rapid rise of the constant for the female takes place within one week between the middle and the end of the

TABLE 6

The data on the weights of brain and body, and of body length according to sex. This table was derived from table 1

BODY		BRAIN		SEX	NUMBER
Weight	Length	Observed	Calculated		
grams	*mm.*	*gram*	*gram*		
10.0	123.	.0776	.0796	Male	5
11.3	133.	.0840	.0844	Male	5
13.2	131.	.0902	.0908	Female	5
13.4	129.	.0934	.0920	Male	5
14.7	135.	.1006	.0960	Male	5
15.9	139.	.1038	.0990	Male	5
16.1	140.	.0990	.0986	Female	5
17.0	135.	.1016	.1006	Male	5
18.1	136.	.0970	.1046	Female	5
18.1	137.	.1030	.1034	Male	5
18.9	141.	.1106	.1056	Female	5
19.5	145.	.1124	.1076	Male	5
20.7	147.	.1108	.1106	Female	5
22.4	151.	.1152	.1136	Female	5
25.5	150.	.1162	.1174	Female	5
25.9	152.	.1183	.1186	Male	3
27.4	159.	.1100	.1216	Female	5
37.8	171.	.1418	.1368	Female	5
					88

month. This period corresponds with the ovulation of the frog. It is, therefore, probable that the marked irregularity of the female constant for March is due to reproduction on the part of the female.

However, as to the manner in which this phenomenon occurs I have as yet a very indefinite conception.

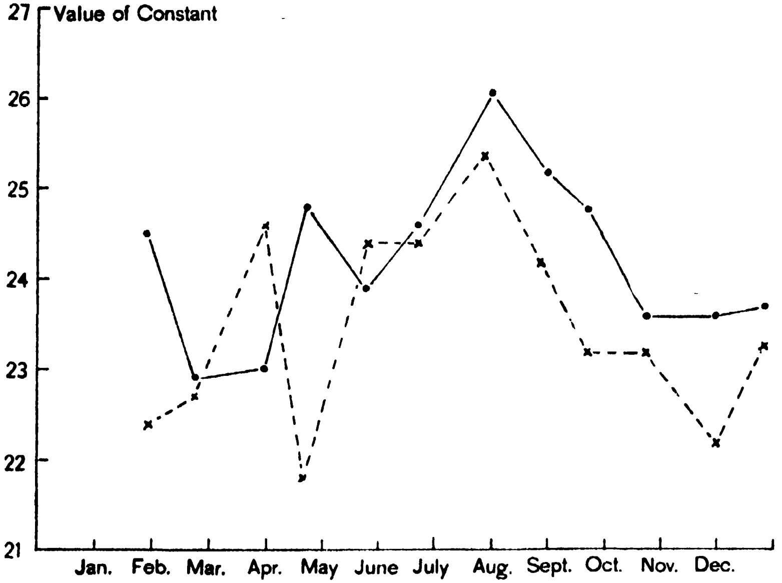


Chart 5 Showing the curve of the constant in the course of a year for both sexes. Male, ————————. Female, – – – – – –.

In the literature on the subject one finds but few attempts at discovering the sex difference of the central nervous system in the lower organisms. Hatai found the water content of the central nervous system of a gray snapper (Neomaenis griseus) to be 77.4 per cent for the male and 78.1 per cent for the female ('18). In my present study the male frog showed 84 per cent of water and the female 84.5 per cent, the result being strikingly similar to that of the above worker.

The sex difference may thus be found not only in the frog, but also in other organisms phylogenetically lower in station.

SEASONAL CHANGES AND SEX DIFFERENCE IN THE NON-PROTEIN NITROGEN OF THE CENTRAL NERVOUS SYSTEM

In a study of the amount of non-protein nitrogen in the central nervous system of the normal albino rat ('18) Hatai concluded: "The number of milligrams of non-protein nitrogen per gram of solid decreases rapidly in the course of the first fifteen days of life. After the fifteenth day, the approximate time when myelin appears, and up to about one hundred and twenty days, the rate of decrease becomes much slower.

TABLE 7

Showing the value of the constant in the two sexes

DATE		SEX AND NUMBER OF TESTS		VALUE OF CONSTANT IN THE TWO SEXES	WATER PER CENT OF BRAIN AND CORD OF BOTH SEXES	
					Brain	Cord
January	27	Female	3	22.4	84.6	80.2
		Male	2	24.5	84.0	80.4
February	22	Female	2	22.7		
		Male	3	22.9		
March	29	Female	2	24.6	83.5	79.9
		Male	3	23.0	83.7	79.8
April	20	Female	2	21.8	84.1	80.2
		Male	3	24.8	84.0	80.4
May	24	Female	3	24.4	85.0	80.3
		Male	2	23.9	84.2	80.5
June	20	Female	3	24.4	84.8	80.0
		Male	3	24.6	83.5	79.8
July	25	Female	8	25.4	84.2	80.0
	31	Male	7	26.1	84.2	79.5
August	25	Female	5	24.2	84.4	81.4
	31	Male	7	25.2	84.3	80.5
September	20	Female	8	23.2	84.4	80.6
		Male	3	24.8	84.3	80.4
October	21	Female	4	23.2	84.4	80.2
		Male	5	23.6	83.8	79.9
November	30	Female	3	22.2	84.6	80.2
		Male	3	23.6	84.4	80.2
December	24	Female	2	23.3	84.6	79.9
		Male	2	23.7	83.6	80.2
Average		Female		23.5	84.5	80.2
		Male		24.2	84.0	80.2

After one hundred and twenty days the change is hardly noticeable." In a similar study on the metabolic activities of the central nervous system of the albino rat the same author noted that the water content decreases with increase of age with an opposite effect on the amount of solids. These studies point to the necessity for a knowledge of the age of the material in a metabolic study of the central nervous system. Although the exact age of the frog is unknown, I have carried out the examination because of the fact that the frogs do not show a noticeable difference of water content according to the age. It is, therefore, to be presumed that the progress of age does not interfere to a noticeable degree with the amount of non-protein.

TABLE 8

Showing the seasonal changes and sex difference in the amount of non-protein nitrogen

SEASON	NUMBER OF TEST	NON-PROTEIN NITROGEN PER 100 GRAM OF BRAIN
		mgm.
Spring	3	182
Summer	2	176
Autumn	2	164
Winter	2	196

The method of the examination is essentially the same as was used in the first and second reports of my study. The results are shown in table 8.

The amount of the non-protein nitrogen is, thus, highest in the winter and decreases gradually through the course of the year, and finally reaches its minimum in the autumn. This is further evidence of seasonal changes in the metabolic activities of the central nervous system. The highest amount of the non-protein nitrogen in the winter is presumed to have a direct bearing on the process of hibernation. The non-protein nitrogen seems to decrease in the following seasons owing to the active metabolic and assimilatory process on the part of the nervous system.

The interpretation has further been confirmed by the following experiment.

Frogs artificially awakened from hibernation by placing them in warm water of 30°C. begin active movements—swimming, embracing, croaking, etc.

These frogs were subjected to the same metabolic studies first, every half-hour and later every hour. The results are given in table 9. The non-protein nitrogen in the test animal is decidedly lower than that in the control. The duration of the time in which the frogs remain does not seem to influence the amount of proportionate decrease. The intensity of the amount of decrease seems to depend more on the character of the stimulation than on its duration (cf. the second part of my study).

TABLE 9

The amount of non-protein nitrogen in the central nervous system during hibernation and during activity

NON-PROTEIN NITROGEN PER 100 GRAM OF BRAIN		
In control (hibernating)	In tests (after awakening)	Time after awakening
mg.	*mg.*	
176	144	30 minutes
	166	1 hour
	164	1 hour and 30 minutes
	153	2 hours
	168	2 hours and 30 minutes
	155	3 hours
	167	4 hours
	146	6 hours
	158 (average)	

As to the sex difference in the amount of non-protein nitrogen, the data on hand at present are too meager to allow any definite conclusion.

However, I have found in the frogs which were examined during the months of spring that while the female shows 184 mg. per 100 grams of brain, the male brain presents the lower value of 181. It would be desirable to follow up the seasonal changes in both males and females, but being unable to do so in the present study I am now attempting further investigation.

BIOLOGICAL INTERPRETATION OF THE SEASONAL CHANGES IN THE RELATIVE WEIGHT OF THE CENTRAL NERVOUS SYSTEM AND THE DIFFERENCE OF METABOLIC ACTIVITIES IN BOTH SEXES

As the frog grows larger the nervous system increases in weight. The increase in weight is due to the enlargement of the cell elements which are always present in the system. In the case of neurones already developed, the enlargement means an increase in the volume of the cell bodies, in the number and size of the dendrites, and in the length and diameter of the axone and its medullary sheath. In the case of the undeveloped neurones, it means a rather rapid acquisition of the branches and medullary sheath, to be followed by the slower changes just described above. In the process of growth the colloid substance imported with the cell body and assimilated increases the volume of solids, the consequence being a decrease of the water content. Thus the amount of water contained in a system is indicative of the growing process of the same organ. It is a fact, proved by Donaldson and others, that in the central nervous system the water content decreases with the growth of the organ.

I have found that the water content of the central nervous system of Rana nigromaculata increases in the summer and decreases in the spring.

This can also be seen in the table of Donaldson's study on the seasonal changes in the relative weight of the central nervous system of the leopard frog. I have shown also that the non-protein content of the central nervous system is found to be highest in the winter and spring, while it is lowest in the summer and autumn.

The water content and the amount of the non-protein nitrogen stand thus in reverse proportion. Therefore, the increase of the non-protein nitrogen should not be regarded as related directly to the growing processes of the central nervous system.

What significance is, then, to be attributed to this seasonal change of the non-protein nitrogen? We know little of the origin of the non-protein substances which are produced by complicated processes, but we have reason to imagine that not all parts of the non-protein nitrogen are contained in the cell body in an assimilated condition.

The non-protein nitrogen contained in the cell body and its prolongations in a state of saturation would flow out in pathological conditions and also under experimental procedures. I believe I have made this clear in my first and second reports and, therefore, the readers are referred to my previous works on this particular question.

The increase of the non-protein nitrogen in the winter and spring is most probably not due to the assimilated substance, but merely due to deposition. Through winter and spring frogs are in hibernation with the exception of a week in March in which they temporarily wake up for ovulation.

In this period of the year they lead a most inactive life, vita minima, and every function is so executed as to sustain their life until the warm season. The nerve cells do not seem to function to the extent of assimilating nutriments to their own substance. It is reasonable, therefore, to conclude that the nerve cells accumulate non-protein nitrogen in the condition of hibernation merely as depositions. In the summer and autumn this is considered as being used up for the assimilatory process as well as for the kinetic activities.

This explanation fits the findings of the seasonal changes in the relative weight of the central nervous system.

The sex difference in the relative weights of the central nervous system offers no less interest. The lower value of the weight for the female is considered to be due to the inactivity of the metabolic process in the central nervous system. This latter condition is again due to the sluggishness of movement on account of hibernation and also to the use of much of the nourishment for the development of the ova.

CONCLUSIONS

1. Donaldson's formula is available not only for the frogs of America and Europe, but for the frogs of Japan as well.

2. The weight of the central nervous system increases according to the increase of the total length and body weight. The increase in the relative weight follows a logarithmic curve.

3. Rana nigromaculata shows regular seasonal changes in the weight of the central nervous system, the same as in American and European frogs. The relative weight is low at the time of emergence, high in the midsummer, and low again at the time of hibernation.

4. Rana nigromaculata shows a marked sex difference in the weight of the central nervous system. It is decidedly higher in the male than in the female.

5. Both the non-protein nitrogen and the water content are higher in the female than in the male.

6. Even the same species of frogs show varied relation in growth according to the difference of the surrounding conditions, such as food, climate, locality, etc.

The writer wishes to express his gratitude to Professor Hatai, whose instructions and cooperation enabled him to furnish this article.

BIBLIOGRAPHY

DONALDSON, H. H. 1902 On a formula for determining the weight of the central nervous system of the frog from the weight and length of its entire body. Decennial publications, University of Chicago, vol. 10, pp. 17–30.

1908 The nervous system of the American leopard frog, Rana pipiens, compared with that of the European frogs, Rana esculenta and Rana temporaria (fusca). Jour. Comp. Neur. Psych., vol. 18, no. 2, pp.121–149.

1911 On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog. Jour. Morph., vol. 22, no. 3, pp. 663–694.

DONALDSON, H. H., AND SHOEMAKER, D. M. 1900 Observations on the weight and length of the central nervous system, and of the legs in frogs of different sizes (Rana esculenta and Rana temporaria). Jour. Comp. Neur. Psych., vol. 10, no. 1, pp. 109–132.

DUBOIS, E. 1898 Ueber die Abhängigkeit des Hirngewichtes von der Körpergrösse bei den Säugethieren. Archiv. für Anthropologie, Bd. 25, S. 1–32.

HATAI, S. 1917 Metabolic activity of the nervous system. I. The amount of non-protein nitrogen in the central nervous system of the normal albino rat. Jour. Comp. Neur., vol. 29, pp. 361–378.

1918 Metabolic activity of the nervous system. II. The partition of non-protein nitrogen in the brain of the gray snapper (Neomaenis griseus) and also the brain weight in relation to the body length of this fish. Jour. Comp. Neur., vol. 29, pp. 41–59.

KOMINE, S. 1919 Metabolic activity of the nervous system. III. On the amount of non-protein nitrogen in the brain of albino rats during twenty-four hours after feeding. Jour. Comp. Neur., vol. 30, pp. 397–410.

1919 Metabolic activity of the nervous system. IV. The content of non-protein nitrogen in the brain of the rats képt in a state of emotional and physical excitement for several hours. Jour. Comp. Neur., vol. 31, no. 2, pp. 69–82.

SNELL, O. 1892 Die Abhängigkeit des Hirngewichtes von dem Körpergewicht und den geistigen Fähigkeiten. Archiv. für Psychiatrie und Nervenkrankheiten, Bd. 23, S. 436–446.

BOTANISCHE BEOBACHTUNGEN IN JAPAN

HANS MOLISCH

Während meiner Tätigkeit an der Tohoku Kaiserlichen Universität in Sendai hatte ich Gelegenheit, sowohl hier als auch an anderen Orten des japanischen Inselreiches, botanische Beobachtungen zu machen. Ein Teil davon soll hier in diesen Blättern fortlaufend veröffentlicht werden.

Die Beobachtungen betreffen sehr verschiedene Gegenstände: anatomische, mikrochemische und physiologische und sollen in der Reihenfolge, wie sie gemacht worden sind, veröffentlicht werden.

I. MITTEILUNG

ÜBER ELAEOPLASTEN BEI BOTRYCHIUM TERNATUM SW. UND OPHIOGLOSSUM VULGATUM L.

MIT EINE FIGUR

In der Abteilung der Pteridophyten wurde bisher das Vorkommen von Elaeoplasten nur bei einer Gattung festgestellt und zwar von Zimmerman[1] im Stengel von Psilotum. Ich bin in der Lage zwei neue Fälle bekannt zu geben. Sie beziehen sich auf Botrychium und Ophioglossum. Es ist sehr auffallend, dass man diese überaus grossen und deutlichen Elaeoplasten bisher übersehen hat.

Der Grund dafür mag vielleicht darin liegen, dass Botrychium den Botanikern nicht besonders leicht zugänglich ist, zumal die Pflanze in botanischen Gärten nicht kultiviert wird. Etwa 20 Minuten Weges entfernt vom botanischen Institute in Sendai wächst Botrychium ternatum Sw. im Schatten riesiger Cryptomerien ziemlich häufig und so hatte ich Gelegenheit, die Pflanze oft zu untersuchen und mir zu verschaffen.

---

[1] Molisch, H., Mikrochemie der Pflanze. 2. Auflage, Jena, 1921, S. 393.

Am deutlichsten ausgebildet und am leichtesten aufzufinden sind die Elaeoplasten in der Epidermis des Blattstiels und der Blattspreite. Die länglichen Oberhautzellen des Blattstiels enthalten je einen eigenartigen farblosen Inhaltskörper, der bei oberflächlicher Betrachtung wie ein im Spiremstadium befindlicher Zellkern mit deutlichem Chromatingerüst aussieht. Allein dieser in der lebenden Zelle sofort sichtbare Inhaltskörper ist nicht der Zellkern. Dieser findet sich gleichfalls in jeder Zelle, ist aber direkt nur selten deutlich zu sehen, wohl aber nach Fixierung und Färbung. Er liegt meist unter dem Elaeoplasten und hat meist eine lang gestreckte, spindelförmige Gestalt (Fig. 1). Die in der Figur gezeichneten Stärkekörner *st* gehören nicht den Epidermiszellen, sondern den unter diesen liegenden Parenchymzellen an. Die Körner liegen hier in Rosenkranz ähnlichen Reihen, in denen sie durch Plasmafäden zusammengehalten werden.

Der auffallende Inhaltskörper besteht aus einem Haufen körniger und schleifenartiger Bildungen von protoplasmaartigem Aussehen, er nimmt oft die ganze Breite der Zelle ein und zeigt folgende Eigenschaften:

In Alkohol (96 prozent) tritt eine Schrümpfung mit teilweiser Lösung ein.

Ist Chlorophyll in der Nähe, wie in den Epidermiszellen des Blattes, so färbt sich der plasmaartige Rückstand infolge der Speicherung des Blattgrüns grün.

In Eisessig treten aus dem Elaeoplasten zahlreiche ölige Kügelchen von verschiedener Grösse aus.

In 10 prozentiger Salzsäure (10 prozent bezogen auf die käufliche Salzsäure) und in 10 prozentiger Schwefelsäure ist die Einwirkung ähnlich wie in Essigsäure, doch erfolgt der Austritt des Öles langsamer.

Das Austreten der Öltropfen lässt sich auch ausgezeichnet mit Chloralhydratlösung (5 Teile Chloralhydrat und 2 Teile Wasser) bewerkstelligen.

In Jod-Jodkaliumlösung treten nach längerer Zeit gleichfalls, wenn auch spärlich, glänzende Tröpfchen aus.

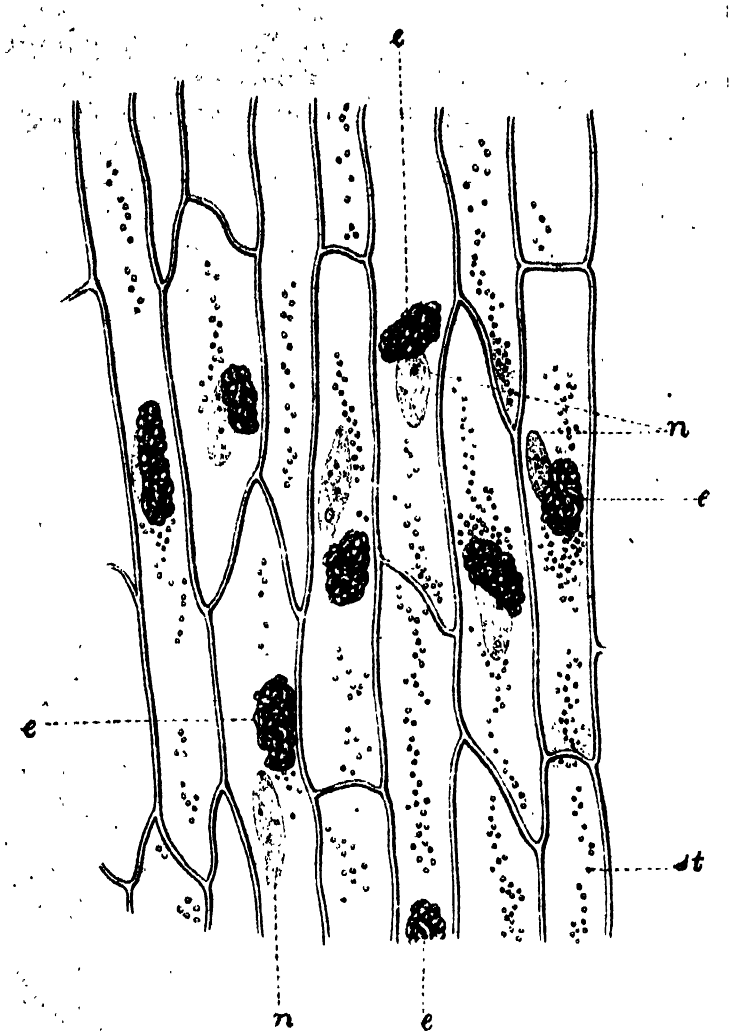


Fig. 1 Botrychium ternatum Sw. Epidermis des Blattstiels. *e*, Elaeoplasten; *n*, Zellkern; *st*, Stärkekörnchen. Vergr. etwa 300.

In Kalilauge (10 prozent) werden die Elaeoplasten zwar desorganisiert, bleiben aber der Form nach deutlich erhalten.

Bei Behandlung mit Ammoniak treten ebenfalls ölige Kügelchen aus und ein schwammiges Netzwerk bleibt zurück. Ebenso wirkt verdünnte Eisenchloridlösung (10 prozent) ein.

In Millons Reagens bleiben sie grossenteils erhalten und färben sich nicht oder ganz schwach rot.

In Sudan III Lösung färben sie sich prächtig rot, wie es ja bei grossen Ölreichtum nicht anders zu erwarten war.

Um diese eigenartigen Inhaltskörper besonders deutlich sichtbar zu machen, eignet sich in ganz ausgezeichneter Weise eine etwa 2 prozentige Lösung von Osmiumsäure. Sie färben sich darin sofort schwarz oder blauschwarz. Bei schwacher Vergrösserung erscheinen sie als kohlschwarze Punkte und jede Zelle zeigt einen solchen.

Nach diesem Verhalten gegenüber den angeführten Reagentien und nach ihrem ganzen Aussehen handelt es sich um einen neuen Fall von Elaeoplasten, um einen Inhaltskörper der Epidermiszellen, der aus einer plasmatischen Grundlage mit eingelagerten Öl besteht.

Dass die Elaeoplasten in der Oberhaut des Blattstiels und der Blattspreite regelmässig vorkommen, wurde bereits hervorgehoben. Im Grundgewebe der genannten Organe sind sie selten, viel schwieriger aufzufinden und im allgemeinen weniger ölreich. Ebenso regelmässsig wie in der Epidermis des gewöhnlichen Laubblattes trifft man sie in der Oberhaut des Fruchtblattes und der Sporangien.

Botrychium virginianum Sw. verhält sich bezüglich der Elaeoplasten und der roten Chlorophyllkörper (S. unten) ebenso wie Botrychium ternatum Sw.

Es ist nicht unwahrscheinlich, dass auch noch andere Botrychium-Arten Elaeoplasten enthalten, ich werde, falls ich Gelegenheit habe, solche Arten zu finden, diese Vermutung genauer prüfen und dann darüber berichten.

Schliesslich sei noch erwähnt, dass die Chloroplasten des basalen Blattstiels, wenn sie starkem Lichte ausgesetzt werden, sich rot färben, wie dies für die Botrychium nahe-stehende Pteridophytengattung selaginella, aber auch für andere Pflanzen so für verschiedene Aloe- und Potamogeton-Arten bekannt ist.[2]

Im Frühjahr 1923 fand ich bei Akabane in der Nähe von Tokyo Ophioglossum vulgatum L. und bei näherer Betrachtung stellte sich heraus, dass auch diese Pflanze sehr grosse, wohl ausgebildete Elaeoplasten besitzt. Sie finden sich in der Epidermis des Blattstiels, der Spreite und des fertilen Blattabschnittes und sind von derselben oder ähnlichen Art wie die von Botrychium, weshalb ich von einer Beschreibung absehe.

Das Vorkommen der Elaeoplasten in den beiden genannten Gattungen ist auch von einem allgemeineren Interesse, denn es stellt ein gutes Beispiel dar, wie sich die Verwandtschaft der Pflanzen auch in gewissen Bestandteilen des Zellinhaltes ausprägen kann.

II. MITTEILUNG

ÜBER EINEN NEUEN PFLANZENFARBSTOFF BEI CLERODENDRON TRICHOTOMUM THUNB.

Gelegentlich eines Spazierganges in der nächsten Umgebung Sendais begegnete ich einem Strauch Clerodendron trichotomum, der durch seine auffallend gefärbten Früchte sofort meine Aufmerksamkeit erregte.

Dem roten, etwas purpurnen, 5-strahligen, bleibenden Kelch sitzt die berlinerblau-gefärbte Beere auf. Dieses eigentümliche, an Früchten so selten anzutreffende Blau erweckte in mir sofort den Verdacht, es könnte sich hier in der Frucht nicht um Anthokyan sondern um einen von diesem Körper verschiedenen Farbstoff handeln.

Die Epidermis der Frucht besteht aus abgerundeten polygonalen Zellen, deren Inhalt unterm Mikroskop tief himmelblau gefärbt erscheint. Dieselbe Farbe zeigt auch die Parenchymzellen des Fruchtfleisches.

Nicht selten findet sich der blaue Farbstoff in den Zellen nicht gelöst, sondern in unregelmässig geformten Schollen vor.

---

[2] Molisch, H., l.c., S. 252.

Der Farbstoff ist leicht in Wasser löslich, langsam löslich in Alkohol, unlöslich in Xylol, Äther, Chloroform und Schwefelkohlenstoff.

Sehr verdünnte Säuren z.B. Essigsäure, Schwefelsäure und Salpetersäure bringen keinen Farbenumschlag nach rot hervor, der Farbstoff kann also kein Anthokyan sein, denn es ist bekannt, dass sich alle Anthokyane mit verdünnten Säuren röten.

Durch sehr starke konzentrierte Säuren wie Schwefel- oder Salpetersäure wird der blaue Farbstoff zerstört und misfärbig.

In sehr verdünnten Alkalien z.B. in Kalilauge oder Ammoniak tritt ein Farbenumschlag nach grün ein, worin unser Farbstoff vielen Anthokyanen gleicht. Konzentrierte Alkalien färben zunächst grün dann gelb oder misfärbig braun und bei Anwendung von Kalilauge in Gewebe schliesslich rötlich.

Sehr verdünntes Eisenchlorid bewirkt sofort einen Farbenumschlag nach schmutziggrün und dieser Farbenton geht nach einiger Zeit in ein misfarbiges Braun über.

Eisensulphat wandelt die blaue Farbe in eine schmutzig grüne um. Nach diesem Verhalten gegenüber Eisensalzen steht der Farbstoff vielleicht den Gerbstoffen nahe.

Leider ist es mir trotz vielfacher Bemühungen nicht gelungen, den Farbstoff zur Kristallisation zu bringen. Das ist mit ein Grund, warum sich über die Natur des Farbstoffes vorläufig nichts Bestimmtes aussagen lässt. Er kann vielmehr jetz hauptsächlich nur durch folgende negative Merkmale charakterisiert werden:

1. Er ist kein echtes Anthokyan, denn er weicht von allen Anthokyanen dadurch ab, dass er bei Behandlung mit Säuren nicht von Blau in Rot umschlägt. Er steht aber wahrscheinlich den Anthokyanen nahe.
2. Er ist kein Phykocyan, denn er besitzt keine Eiweissnatur und zeigt keine Fluoreszenz.
3. Er ist nicht Indigo, denn er weicht von diesem Farbstoff gänzlich ab und lässt sich auch nicht sublimieren.

Die von mir als 'Pseudoindikane'[3] bezeichneter Chromogene sind dadurch ausgezeichnet, dass sie erst postmortal einen blauen Farbstoff liefern, während der blaue Farbstoff in der Beere von Clerodendron trichotomum schon in der lebenden Zelle vorhanden ist.

Der in der Kelchoberhaut vorhandene rote Farbstoff zeigt die Eigenschaften des Anthokyans im Gegensatze zu dem Farbstoff der Frucht. Das Kelchblatt ist anatomisch dadurch ausgezeichnet, dass unmittelbar unter der oberen Oberhaut gegen die Mittelrippe zu sich eine meist einschichtige Lage von flachen, wellig kontourierten Steinzellen, deren Wände verholzt sind, vorfindet, ein für ein Kelchblatt gewiss sehr seltener Fall.

Mit diesen spärlichen Angaben muss ich mich vorläufig bescheiden, sie verfolgen lediglich den Zweck, die Aufmerksamkeit auf diesen neuen Pflanzenfarbstoff zu lenken und vielleicht einen Makrochemiker zu veranlassen, ihn weiter zu studieren.

Schliesslich will ich noch einen zweiten Fall eines vom Anthokyan abweichenden blauen Farbstoffes erwähnen.

Die Narbe der Blüte von Monotropa uniflora L. ist blau gefärbt und diese Färbung verdankt sie einem himmelblauen Farbstoff, der in den subepidermalen Parenchymzellen des Stigma gelöst vorkommt. Er hat mit dem Farbstoff von Clerodendron in so ferne Ähnlichkeit als auch er mit Säuren (Salzsäure, Essigsäure, etc.) nicht rot wird.

---

[3] Molisch, H., Mikrochemie der Pflanze. 2. Auflage, Jena, 1921, S. 239.

ON THE AGGLUTINATING SUBSTANCE OF THE EGG OF THE STARFISH, ASTERINA PECTINIFERA

SHICHIROKU NOMURA

Recently the egg secretions of starfishes and sea-urchins have been studied by various investigators, and it has become evident that the egg secretions of these animals contain a substance or substances, which are seemingly responsible for production of the so-called 'agglutination' phenomena upon the spermatozoa of the same species. During my stay (July to September, 1922) at Asamushi, Aomori Prefecture, I was fortunate in being able to study the phenomena just mentioned on the starfish, Asterina pectinifera (Müller and Troschel).

In this paper I shall present briefly a preliminary account of the general behavior of the spermatozoa when mixed with the egg water. Detailed studies will follow in the near future.

Agglutination. The observations were made, for the most part, under the microscope. If the spermatozoa are brought in contact with the egg water, viz., sea-water which contains egg secretions, they are highly activated. The spermatozoa thus activated form in a few minutes numerous agglutination masses resulting from the adherence of one to another by the head, which seems to have become sticky and swollen, while the tail remains free in most spermatozoa. This seems to be the characteristic feature of iso-agglutination as compared with hetero-agglutination, where not only the heads become sticky and swollen, but the tails also adhere. Swelling of the sperm head was observed also by Lillie ('19, p. 120) and Sampson ('22, p. 277). The agglutination masses are at first composed of only several spermatozoa, but sooner or later the number may increase to more than one hundred.

The results of the observations are summarized and presented in the tables. It is to be understood that all observations included under the same data were carried out with materials which were obtained from the same source. Those preparations which were mounted on slide glasses almost simultaneously and observed side by side are connected with a brace. From table 1 it will clearly be seen that agglutination reaction invariably occurs on the slide upon which the egg water was placed, but always fails where it was absent. It is, therefore, evident that the egg water of Asterina pectinifera contains a substance which agglutinates the spermatozoa of the same species. Loeb reports that the sperm of Asterina gave agglutination reaction neither with their own egg water nor with that of sea-urchins (Loeb, '14, p. 125), but he does not give the name of the species under consideration.

TABLE 1

DATE OF EXPERIMENT	NO. OF SLIDE GLASS	EGG WATER		SEA-WATER IN DROPS	SPERM SUSPENSION		REACTION (AGGLUTINATION)
		Relative concentration	Quantity in drops		Relative concentration	Quantity in drops	
17/VIII	I	1	Few	0	$\frac{1}{10}$	Few	(+) (+)
	II	1	Few	0	$\frac{1}{10}$	Few	(+) (+)
	V		0	1	1	3	(—)
	VI	$\frac{1}{50}$	1	0	1	3	(+) (+)
	VII	$\frac{1}{500}$	1	0	1	3	(+) (+)
	VIII		0	1	1	3	(—)
	IX	$\frac{1}{500}$	1	0	1	3	(+) (+)
	X	$\frac{1}{5000}$	1	0	1	3	(+)
	A	$\frac{1}{2}$	1	0	1	5	(+) (+)
	B		0	0	1	5	(—)
21	IV	$\frac{1}{2}$	5	0	1	5	(+)
	V	$\frac{1}{2}$	3	2	1	5	(+)
	VI		0	5	1	5	(—)
2/IX	V	$\frac{1}{50}$	1	0	Dilute	1	(+)
	VI	$\frac{1}{50}$	2	0	Dilute	1	(+)
	VII	$\frac{1}{50}$	1	0	Concentrated	1	(+) (+)
	VIII		0	1	Concentrated	1	(—)

I may add, moreover, that Asterina egg water seems also to produce hetero-agglutination of the spermatozoa of Strongylocentrotus tuberculatus Lamk. (Toxocidaris tuberculatus Mortensen) (Mortensen, '14) and of Laonome.

Effective concentration. The experiments of 17/VIII (table 1) were carried out to determine the minimum concentration of the egg water necessary for production of agglutination. The original egg water was diluted to various concentrations, and it was found that agglutination was perceptible even with such a low concentration as 1/5000 of the original. Similar remarkable effectiveness of agglutinating substance in low concentration was observed also by Lillie ('19, p. 117) and others.

TABLE 2

DATE OF EXPERIMENT	NO. OF HOLLOW GLASS	SPERM SUSPENSION (10/VIII), DILUTED	MATERIAL FOR TEST OR CONTROL	REACTION (AGGLUTINATION)	
11/VIII	I	0.05 ccm.	A few eggs, 5 times washed with sea-water.	(+)	Spermatozoa crowded enormously over egg surface.
	II	0.05 ccm.	Primary egg water 2 drops	(+)	
	III	0.05 ccm.	Secondary egg water 2 drops	(+)	
	IV	0.05 ccm.	Tertiary egg water 2 drops	(+)	
	V	0.05 ccm.	Quaternary egg water 2 drops	(+)	
	VI	0.05 ccm.	Sea-water 2 drops	(—)	

The effect of more diluted egg water was not tested.

In the experiments given in table 2, eggs left over night in a test-tube were washed five times with sea-water. The amount of water used for this purpose was nearly ten to twenty times that of the eggs in each case.

The eggs, even after the fifth washing, were able to attract and agglutinate the spermatozoa. It is interesting to note also that the egg water obtained from even the fourth washing was able to produce agglutination.

The eggs did not reach full maturity during my stay at Asamushi. No eggs were fertilizable, but they produced the agglutinating substance. Occasionally small egg cells which neither attracted nor agglutinated the spermatozoa were met with, while large eggs apparently possessed the power to do so. The eggs seem to begin to produce the substance a short time before or during the period of growth. Von Dungern also reports that unripe ova of starfish produce agglutinating substance ('02, p. 41).

TABLE 3

DATE OF EXPERIMENT	NO. OF SLIDE GLASS		MATERIAL FOR TEST		SPERM SUSPENSION		SEA-WATER IN DROPS	RESULT (AGGLUTINATION)
			Relative concentration	Quantity in drops	Relative concentration	Quantity in drops		
1/IX	VIII	A	E. W.	2	1	1	0	(+)
		B	E. W.	2	1	1	0	(+)
	IX	A	E. W.	1	1	1	0	(+)
		B		0	1	1	1	(—)
	X	A	E. W.	1	1	1	0	(+)
		B		0	1	1	1	(—)
	XI	A	B. F.	1	1	1	0	(—)
		B		0	1	1	1	(—)
	XII	A	L. E.	1	1	1	0	(—)
		B		0	1	1	1	(—)
	XIII		$\frac{1}{10}$ E. W.	1	1	1	0	(+)
	XIV		$\frac{1}{10}$ E. W.	1	1	1	0	(+)
	XV		$\frac{1}{2}$ B. F.	1	1	1	0	(—)
	XVI		$\frac{1}{3}$ L. E.	1	1	1	0	(—)
	XVII			0	1	1	1	(—)
	XVIII			0	1	1	0	(—)
2	I		E. W.	1	1	1	0	(+)
	II		B. F.	1	1	1	0	(—)
	III		L. E.	1	1	1	0	(—)
	IV			0	1	1	1	(—)

Abbreviations: *E.W.*, egg water; *B.F.*, body fluid; *L.E.*, liver extract.

Tissue specificity. The tissue specificity of the agglutinating substance is known in some other forms. To ascertain this point in the starfish, the coelomic or body fluid and the extract of the liver or pyloric caecum of the same specimen, the eggs of which produced the agglutination, were tried on the spermatozoa of the same species. The results of the experiments are given in table 3. In the series of experiments

VIII–XII on September 1st, the two separate mountings A and B were prepared on each slide and observed side by side.

In the preparations where egg water was present, agglutination reaction was evident, but in others where tissue extract alone was present, it was always negative. This naturally leads us to conclude that the agglutinating substance is present in the eggs, but absent, so far as the observations go, in other tissues.

This work was begun at the suggestion of Professor Hatai, to whom I wish to express by sincere thanks for his kind advice on various points and for his review of the manuscript.

LITERATURE CITED

DUNGERN, EMIL VON 1902 Neue Versuche zur Physiologie der Befruchtung. Zeitschr. f. allgem. Physiol., Bd. 1, S. 41.

GOTO, SEITARO 1914 A descriptive monograph of Japanese Asteroidea. Jour. Coll. Sci. Imp. Univ. Tokyo, vol. 29, art. 1.

LILLIE, FRANK RATTRAY 1913 Studies of fertilization. V. The behavior of the spermatozoa of Nereis and Arbacia with special reference to egg extractives. Jour. Exp. Zoöl., vol. 14.

1919 Problems of fertilization. Univ. Chicago Science Series.

LOEB, JACQUES 1914 Cluster formation of spermatozoa caused by specific substances from eggs. Jour. Exp. Zoöl., vol. 17, no. 1, pp. 123–140.

MORTENSEN, TH. 1914 On the development of some Japanese echinoderms. Annot. Zool. Japon., vol. 8, part V.

SAMPSON, M. M. 1922 Iso-agglutination and hetero-agglutination of spermatozoa. Biol. Bull., vol. 43, no. 4, pp. 267–284.

ALLOLOBOPHORA FOETIDA (SAV.) IN NORTH JAPAN

KIICHIRO SASAKI

At the Biological Institute of the Tohoku Imperial University several investigators are using a certain species of Allolobophora as material for various research purposes. It thus became necessary to determine the specific name of the earthworm, and I have undertaken this investigation under the direction of Assistant Professor E. Nomura.

The earthworm under consideration is called 'Shimamimizu,' or 'striped worm,' and anglers use it very commonly, because it is considered to be the best bait for fishing. It is usually found abundant in the manure piles of cattle feces, more or less decayed. These worms can be kept very easily if, for instance, they are placed in the corner of a garden and fed with rice paste.

This worm, when it is alive, has a very characteristic coloration; each dorsal segment shows three stripes, light red in front, deep red mixed with purple in the middle, followed again by light red, with the exception of segments IX, X, and XI, and of the clitellar portion, in which the coloration is somewhat weaker. The ventral is nearly the same as the dorsal, only somewhat whiter. The body of the worm is flat when contracted, but becomes cylindrical when relaxed.

In formalin the color gradually fades away, but in alcohol, if the worms are previously fixed in acetic sublimate, it turns to a very light blackish yellow ocher and the striation disappears.

The chief specific characters are as follows:

Clitellum, XXVI–XXXII (7 segments), it has, however, a tendency to cover several anterior segments, also.

First dorsal pore, IV/V.

Setae, strictly paired, on the middle striation.

Ovary, XIII, 1 pair.

Testis, X, XI, 2 pairs.

Male pore, XV, 1 pair.

Sperm sac, IX, X, XI, XII.

Spermathecae, IX, X, 2 pairs.

Spermathecal openings, IX/X, X/XI (dorsal openings).

Length, 86 mm. (76–106 mm.), average of 15 specimens.

Breadth, 4 mm. (3–4, 3 mm.), average of 15 specimens.

Number of segments, 98, average of 15 specimens.

The specific description given above thus agrees with those given by Beddard of Allolobophora foetida (Sav.) in the Monograph of the Order Oligochaeta (1895), and also agrees with Eisenia foetida (Sav.) of Michaelsen (Das Tierreich, Bd. 10, 1900).

So I conclude that the earthworms which we are using in our laboratory are Allolobophora foetida (Sav.).

I am as yet unable to give the exact distribution of this species in Japan, but I can state that it must be very common along the Pacific coast of north Japan, since I found this species among the collections from Tokyo, Sendai, Morioka, and Aomori. It thus seems highly probable that this species is very widely distributed in Japan. It is strange that I have not yet found specimens of Allolobophora japonica Michaelsen. It is interesting to note also that this cosmopolitan species is found in Japan, and despite its world-wide distribution the worms are practically immune to climatic or geographical variations which commonly occur in most other animals.

ZUR KENNTNIS DER KEIMENTWICKLUNG BEI SARGASSUM

MASATO TAHARA

MIT VIER ABBILDUNGEN IM TEXT

Es ist auffallend, dass während eingehende Beobachtung über die Befruchtung und Keimentwicklung bei Fucus schon in Hülle und Fülle durchgeführt sind, Sargassum bis jetzt in dieser Hinsicht nur dürftig behandelt erscheint. Um einige Beiträge auf diesem Gebiete zu liefern, begab ich mich in März, 1920, nach Misaki zur Biologischen Station der Tokio kaiserlichen Universität und während des dreitägigen Aufenthaltes beschäftigte ich mich mit der Sammlung des Materials. Nach den Erfahrungen der früheren Jahren wurde die Fixierung des Materials meistens in der Nacht durchgeführt, denn am Tage fixiertes Material dürfte die Teilungsfiguren so gut wie gar nicht zeigen. Um das Material lückenlos zu machen, sammelte ich es je einmal in jeder Stunde und die ganze Nacht hindurch. Doch war das Material für die Untersuchungen über die Befruchtung nicht geeignet. Daher wird hier nur die Embryoentwicklung dieser Alge behandelt werden.

An der Küste von Misaki wuchern verschiedene Arten von Sargassum sehr üppig. Zu meiner Untersuchung wählte ich aber nur eine Art, nämlich Sargassum enerve, Ag. Die folgende Schilderung bezieht sich daher ausschliesslich auf diese Alge. Als Fixierungsmittel benutzte ich Chromessigsäure von folgender Zusammensetzung:

Gesättigte wässerige Lösung von Chromsäure...	1 cc.
Seewasser	100 cc.
Eisessigsäure	1 cc.

Die Schnitte von 15-25 μ Dicke wurden teils mit Heidenhains Hämatoxylin und teils mit Safranin-Lichtgrün gefärbt. Die letzte Methode habe ich lieber benutzt, denn die erstere erschwert die Beobachtung einigermassen, indem so wohl Chromatophoren als auch Kernsubstanz mit dem Farbstoffe intensiv tingiert werden. Dagegen werden mit Safranin die Chromatophoren nicht gefärbt.

Wie schon wohl bekannt, hat das Oogonium von Sargassum acht Kerne im früheren Stadium seiner Entwicklung, obwohl es nur einem einzigen Ei in sich auszubilden bestimmt ist. Diese acht Kerne dürften aber mit Recht alle Eikerne bezeichnet werden, weil sich acht funktionierende Eier tatsächlich bei Fucus in einem Oogonium entwickeln. Seit der Arbeit von Oltmanns nimmt man bloss an, dass die überzähligen sieben Kerne sämtlich früher oder später vernichtet werden. In der im Jahre 1910 erschienenen Abhandlung Nienburgs über Cystosira und Sargassum wird dieser Gedanke wieder geltend gemacht. Aber er berichtet hierüber eine merkwürdige Erscheinung.[1] Er schreibt:

> Ich habe nur noch einen abnormalen Fall (bei Cystosira) zu erwähnen. Es kommt manchmal vor, dass man in einem Ei zwei Kerne findet. Ich habe dann niemals mehr als sechs degenerierte gefunden, so dass es wahrscheinlich ist, dass die beiden Kerne Oogonkerne vorstellen, die nicht ausgewandert sind. Es ist allerdings möglich, dass sie durch Teilung aus dem ursprünglichen Eikern hervorgegangen sind, denn die Zählung der degenerierten Kerne ist nicht genau durchzuführen, da beim Färbeprozess häufig einzelene von ihnen fortgespült werden. Ueber das weitere Schicksal dieser doppelkernigen Eier habe ich nichts Sicheres ermitteln können.

Bei meiner Untersuchung über die Embryoentwicklung von Sargassum enerve ist es mir aufgefallen, dass das Oogonium mit zwei intacten und sechs degenerierten Kernen ganz allgemein ist. Ich habe nie ein Oogonium mit einzigem Eikerne gesehen. Ich werde das unten noch etwas näher beschreiben.

---

[1] Nienburg, W., Die Oogonentwicklung bei Cystosira und Sargassum (Flora, Neue Folge, Bd. 1, 1910), S. 170.

Im früheren Stadium der Entwicklung des Oogoniums häufen sich zahlreiche Chromatophoren dicht um die acht Eikerne. Dann entsteht ein heller Hof in der nächsten Umgebung jedes Kerns. Dieser Hof ist ganz frei von Chromatophoren. Die sechs von den acht bisher ganz normal aussehenden Kerne fangen nun an zu degenerieren (Fig. 1,a). Die Ausstossung der degenerierten Kerne, welche bei den meisten Fucaceen platzgreift, findet bei Sargassum nicht statt. Die Nukleolen der degenerierten Kerne lassen sich im Cytoplasma von Oogonium lange beobachten. Die intact bleibenden zwei Kerne weichen bald auseinander nach den entgegengesetzten künftigen Polen des Keimlinges (Fig. 1,b). Der nach unten schreitende Kern erreicht die aüsserste Grenze des Poles. Die Scheidewand zwischen diesen zwei Kerne wird lange nicht aussgebildet. Daher sehen wir eine Zeitlang die zwei Kerne ganz frei in der gemeinsamen Plasmamasse. Auch Nienburg beobachtete seinerzeit denselben Zustand des Oogoniums bei Sargassum, er hatte jedoch darüber eine ganz andere Ansicht als ich.[2] Er schreibt:

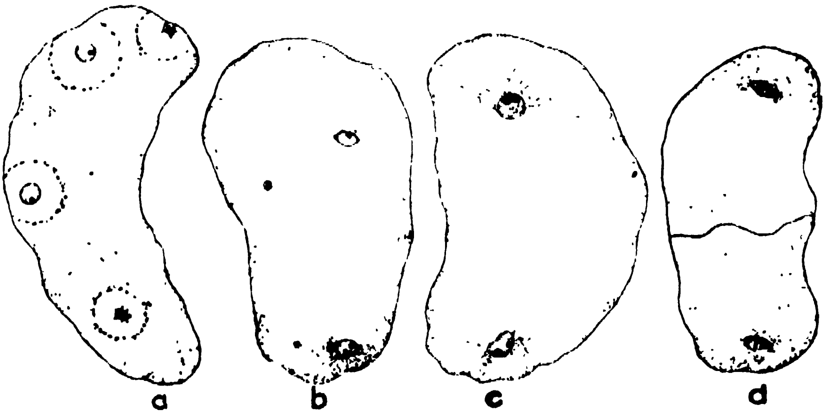


Fig. 1 Keimentwicklung von Sargassum enerve. a, Ei mit zwei intakten und sechs schon degenerierten Kernen. b, Noch späteres Stadium. Nukleolen von den degenerierten Kernen noch bemerkbar. c, Zwei Eikerne in Prophase der Teilung. Plasmastrahlung sehr deutlich. d, Die ersten Kernteilungen in Keimlinge.

---

[2] A. a. O., S. 176.

Ich habe hier bei allen älteren Oogonium, in denen sich der Eikern bereits ausgebildet hatte, an einer bestimmten Stelle der Wandung sich stark tingierende Plasmamassen gesehen, in denen manchmal noch Kerne nachgewiesen werden konnten. Da ich an anderen Stellen der Wandung niemals degenerierte Kerne habe liegen sehen, so nehme ich an, dass sie mit den erwähnten Plasmamassen zusammen ausgestossen werden. Diese werden später wahrscheinlich wieder resorbiert.

Der untere Pol des Keimlinges ist die Stelle, wo sich Rhizoiden später entwickeln, und die Plasmamasse ist merklich dichter in ihrer Konsistenz und färbt sich sehr stark. Aber es handelt sich hier nicht um eine Degeneration des Plasmas, weil der Kern in diesem Bezirke in beinahe gleichen Schritten mit dem oberen Kerne eine mitotische Kernteilung immer ganz regelmässig durchführt (Fig. 1,d).

Wie oben gesagt, liegen die zwei intakten Eikerne zuerst in der gemeinsamen Plasmamasse. Mit dem Fortschritte der Entwicklung wachsen sie immer mehr und endlich kommt um jeden dieser Kerne zur Ausbildung einer sehr deutlichen strahlige Struktur des Plasmas. Diese Struktur findet immer auf der nach dem Pol gerichteten Seite beschränkt, und zwar ist sie besonders in der Umgebung des oberen Kernes (Fig. 1, c und d). Diese zwei Kerne haben sehr häufig je zwei oder drei Nukleolen, was auf eine vorhergegangene Verschmelzung von Ei- und Spermakern hindeuten könnte. Aber im achtkernigen Stadium des Oogoniums beherbergen merkwürdigerweise einige von den Kernen schon zwei oder drei Nukleolen. Bevor die ersten Kernteilungen in Keimlinge stattfinden, bildet sich eine Scheidewand etwa in der Aequatorialebene des Keimlinges aus. In der Prophase der Kernteilung dringen feine Fädchen in den Kern hinein und die bisher sehr deutliche Strahlung des Plasmas erlischt (Fig. 1,d). In der Telophase findet man zwei winzige Kerne neugebildet, um welche die Strahlungen, die Vorbereitung für die zweite Teilung, wieder klar hervortreten (Fig. 1,d). Es wäre ein interessantes Problem, die Chromosomenzahl dieser beiden Kerne zu vergleichen. Leider gelang es mir aber nicht dies durchzuführen, weil die Chromosomen von Sargassum sehr klein und unter-einander dicht gedrängt sind. Die weitere Entwicklung des Keimlinges habe ich schon früher behandelt[3] und es scheint mir daher überflüssig, neuerdings darauf einzugehen.

Kurz zusammengefasst: Bei Sargassum enerve beteiligen sich zwei Eikerne an der Ausbildung des Keimes und es liegt die Vermutung sehr nahe, dass diese Erscheinung unter den gesammten Arten von Sargassum und dessen verwandten Gattungen z.B. Cystophyllum, Cystosira u.a. allgemein verbreitet ist.

Bekanntlich entstehen gewisse Zwillinge durch frühzeitige Verschmelzung zweier Eizellen. Wenn meine obige Beobachtung richtig ist, so könnte man, in der Entwicklung von Sargassum ein Analogon zu dieser Zwillingsbildung erblicken, weil sich die zwei Eikerne von Sargassum immer in der Keimentwicklung beteiligen.

[3] Tahara, M., Oogonium liberation and embryogeny of some fucaceous algae (Journal of the College of Science, Imp. Univ. Tokyo, vol. 32, 1913).

76 Proben von geschlachtetem Rind auf spontanes Leuchten geprüft und zwar 48 Rindfleisch, 10 Kalbfleisch- und 18 Leberproben. Hierbei ergab sich, dass von den gesamten Objekten 48 Prozent leuchteten und zwar:

Rindfleisch	52	Prozent.
Kalbbeisch	50	„
Rindsleber	39	„

Als ich später meine Methode verbesserte und die Fleischstücke in 3 procentige Kochsalzlösung einlegte (siehe weiter unten) fiel das Ergebnis noch günstiger aus, denn von den gesamten, nach der „Salzwassermethode" geprüften Fleischstüken (Rind- und Pferdefleisch) leuchteten nicht weniger als ungefähr 87 Prozent und zwar

von den Rindfleischproben	89	Prozent.
„ „ Pferdefleischproben	65.5	„

Die Ursache des Leuchtens ist nicht etwa das der Zersetzung anheimfallende Fleisch sondern, wie lange bekannt, ein leuchtender Spaltpilz und, wie ich durch viele Reinkulturen feststellen konnte, immer ein und derselbe Spaltpilz, das Bacterium phosphoreum (Cohn) Molisch.

Als ich meine Versuche auch auf Wien ausdehnte, fand ich, dass das Leuchten des Fleisches seltener auftritt als in Prag; es mag vielleicht mit der verschiedenen Behandlung des Fleisches oder mit dem beim Waschen und Besprengen der Fleisches verwendeten Wasser zusammenhängen. In Prag war dies ein ungemein weiches, fast mooriges Wasser, in Wien hingegen ein ziemlich hartes, kalkreiches.

II.

Bei meiner Tätigkeit an der Universität in Sendai interessierte mich neuerdings dieser Gegenstand. Ich legte mir die Fragen vor:

Kommt das spontane Leuchten des Schlachtviehfleisches auch in Japan vor? Und wenn, ist es häufig oder selten? Ruft hier derselbe Spaltpilz das Leuchten hervor wie in Europa oder ist es ein anderer?

Die Methodik, die ich zur Feststellung des Leuchtens angewendet habe, war dieselbe, die sich schon im meiner früheren Praxis bewährt hatte. Es wurden während der kalten Jahreszeit (Dezember-Februar) Fleischstücke im Fleischerladen gekauft, sofort ins Laboratorium gebracht, in eine Petrischale gelegt, mit einer 3 prozentigen Kochsalzlösung in gewöhnlichem Wasser übergossen und, um das Verdampfen der Flüssigkeit und eine Koncentrationsänderung zu verhindern, mit einer Deckelschale bedeckt. Das Fleisch darf nicht in der Salzlösung untergetaucht sein, sondern muss grossenteils aus der Lösung in die Luft ragen. Das Fleisch wird in einem wenig geheizten Zimmer bei einer Temperatur von etwa 10-15°C im diffusen Licht oder im Finstern aufgestellt. Beobachtet wird in der Nacht. Will man schwaches Leuchten nicht übersehen, so muss man mit möglichst ausgeruhtem Auge beobachten. Man muss etwa $\frac{1}{4}-\frac{1}{2}$ Stunde im Finstern verweilen, falls einem schwaches Licht nicht entgehen soll. Kommt man hingegen aus der Tageshelle in die Dunkelkammer, so ist das Auge ermüdet und wenig empfindlich für geringe Helligkeiten. Solche können dann leicht übersehen werden. Ungemein empfindlich aber für Spuren von Licht ist das menschliche Auge, wenn man in der Nacht erwacht und unmittelbar darauf mit völlig dunkeladaptiertem Auge im Finstern beobachtet. Ich bewahre die Fleischproben zu diesem Zwecke in meinem Schlafzimmer. Ich mache absichtlich auf diese Umstände aufmerksam, weil dem Beobachter sonst leicht schwächeres ja mitunter sogar starkes Leuchten entgehen kann.

Die Versuche wurden in Sendai zuerst mit Rindfleisch gemacht. Ich war überrascht schon bei der ersten Probe nach 3 tägigem Liegen das Leuchten feststellen zu können. Das Licht erscheint in Form kleiner leuchtender Punkte. Nach und nach können sich diese zu grösseren Inseln vereinigen, ja in manchen Fällen kann die ganze Oberfläche leuchten.

Unmittelbar nach dem Einkaufen beim Metzger leuchtet das Fleisch nur in seltenen Fällen, wenn es aber unter den früher angegebenen Umständen in der Kochsalzlösung liegt, erscheint häufig das Licht.

Das Leuchten beginnt gewöhnlich am 2-3. Tage und dauert dann 1-3 Tage, bei hoher Sommertemperatur aber viel kürzere Zeit, da sich dann andere Bakterien im Kampfe ums Dasein breitmachen, die stinkende Fäulnis einleiten und damit die Leuchtbakterien in kurzer Zeit so überwuchern, dass das Licht verschwindet.

Um mich zu überzeugen, ob die Lichtentwicklung am Fleische vielleicht nur gerade auf den Fleischerladen beschränkt war, von dem ich das Fleisch zuerst holte, wurde später immer von einem andern Laden Fleisch gekauft, aber trotzdem zeigte sich, wenn auch nicht immer, so doch recht häufig Leuchten.

Im ganzen habe ich bisher 20 Rindfleischproben geprüft und bei 11 in mehr oder minderem Grade Licht beobachtet. Das sind 55 Prozent.

Nachher untersuchte ich Schweinefleisch und fand es zwar hier nicht so häufig leuchtend aber mitunter so auffallend intensiv, dass man das Licht mit wohl ausgeruhtem Auge auf 10 Schritte noch bequem wahrnehmen konnte. In solchen Fällen leuchtete nicht nur das Fleisch sondern auch die Kochsalzlösung.

Ich untersuchte vom Schweinefleisch verschiedener Läden im ganzen 20 Proben und zwar in 3 Fällen mit positiven Erfolge. Das waren alo 15 Prozent.

Vergleichsweise machte ich sowohl mit Rind-als auch mit Schweinefleisch Versuche ohne Kochsalzlösung. Es wurden dann die eben beim Fleischer eingekauften Fleischstücke einfach ohne Kochsalzlösung in die Petrischale eingelegt. Wie schon früher in Europa habe ich auch hier in Sendai gefunden, dass das Leuchten bei dieser „Luftmethode" im Gegensatze zu der „Salzwassermethode" zwar auch auflritt aber seltener. Das kommt daher, weil die Bakterie, die das Leuchten des Fleisches verursacht, halophil ist und durch Kochsalz in ihrer Entwicklung wesentlich gefördert wird.

Durch das mitgeteilte Ergebnis war nun die erste Frage, die ich mir gestellt habe, gelöst. Die Antwort lautet kurz: Das in Sendai zum Verkaufe dargebotene Schlachtviehfleisch vom Rind und vom Schwein kann, wenn es nicht schon leuchtend ist, in vielen Fällen leuchtend gemacht werden, wenn man es mit 3 prozentiger Kochsalzlösung benetzt im feuchten Raume liegen lässt.

Nach Feststellung dieser Tatsache ging ich daran, zu prüfen, ob die Bakterie, die in Sendai die Lichtentwicklung am Fleische hervorruft, identisch ist mit derjenigen, die in Europa das Lichtphaenomen am Schlachtviefleisch veranlasst, nämlich mit Bacterium phosphoreum (Cohn) Molisch.

Es wurden daher mehrmals Reinkulturen von verschiedenen Fleischproben hergestellt und dabei ergab sich, dass in allen Fällen stets eine und dieselbe Bakterie als Photobakterie isoliert werden konnte und zwar die vorhin erwähnte. Eine genaue

Beschreibung dieser Bakterie habe ich seinerzeit in meiner Abhandlung[1] über das Leuchten des Fleisches gegeben, weshalb ich darauf verweise.

Aus dem Gesagten erhellt, dass die genannte Photobakterie sich ebenso wie in Europa auch in Sendai und wohl höchstwahrscheinlich in andern Städten Japans in den Schlachthäusern und den Fleischläden eingebürgert hat und an diesen Orten auf das frisch eingebrachte Fleisch immer vom neuem übertragen wird.

Es sei ausdrücklich betont, dass diesem Spaltpilz keine pathogenen Eigenschaften zukommen und dass das Fleisch selbst in leuchtendem Zustande, wenn es noch keinen üblen Geruch hat, genussfähig ist. Es verhält sich in dieser Beziehung das Fleisch genau so wie die Meeresfische, die ja, wie seit langem bekannt, kurze Zeit nach dem Absterben gleichfalls ;in folge von Photobakterienbesiedlung leuchtend werden. Die meisten Fische, die auf dem Fischmarkt zum Verkaufe bereit liegen, leuchten,[2] sind aber trotzdem genussfähig. Erst wenn andere Bakterien über die Leuchtbakterien die Oberhand gewinnen, diese verdrängen und die stinkende Fäulniss einleiten, dann dürfen solche Fische nicht mehr gegessen werden.

III.

Zum Schluss möchte ich noch eine Bemerkung machen, die in allgemein biologischer Beziehung von Interesse ist. Sie betrifft die sehr häufige weite Verbreitung und den Kosmopolitismus der Mikroorganismen. Ich habe seinerzeit die farblosen Schwefelbakterien im Wasser aus dem Hafen von Triest (früher Oesterreich. jetzt Italien) untersucht und konnte unter andern von neuen Arten auffinden: Thiothrix annulata Molisch, Thiothrix marina Molisch, Beggiatoa marina Molisch, Bacterium Bovista Molisch, Bacillus thiogenus Molisch, Spirillum punctatum Molisch und ausserdem eine neue Bakterie die in auffallend langen unbeweglischen Fäden ohne Schwefelkörnchen auftritt, nämlich Chlamydothrix longissima Molisch.[3]

Als ich die Schwefelbakterien, die im Seewasser von Matsushima

---

[1] Molisch H., Ueber das Leuchten des Fleisches, insbesonders toter Schlachttiere. Botanische Zeitung. 1903.

[2] Molisch H., Leuchtende Pflanzen. 1. c. p. 79.

[3] Molisch H., Neue farblose Schwefelbakterien. Centralblatt f. Bakteriologie etc. Bd. 33. 1912 p. 55.

(Pacific bei Sendai in Japan) auftreten, näher studierte, fand ich fast alle die genannten Bakterien auch hier vor.

Auch die in Europa allenthalben vertretenen Süsswasser-Beggiatoa- und Thiothrix-Arten kommen in den mit schwarzen Schlamm erfüllten längs der Häuser oberflächlich verlaufenden Abzugskänälen häufig vor. Gelegentlich meines Aufenthaltes auf Java (1897/98) konnte ich mich von dem Vorkommen der in Europa so allgemein verbreiteten Schimmelpilze: Mucor Mucedo, M. racemosus, Rhizopus nigricans, Penicillium glaucum und von dem Auftreten der Eisenbakterie Leptothrix ochracea überzeugen. Auch aus der inhaltsreichen Arbeit Saitos[1] geht hervor, dass die in Europa aufgefundenen, weit verbreiteten Schimmelpilze auch in Tokyo regelmässig anzutreffen sind. Es seien nur die am häufigsten vorkommenden genannt: Cladosporium herbarum, Penicillium glaucum, Epicoccum purpurascens, Aspergillus glaucus, A. nidulans, Catenularia fuliginea, Mucor racemosus, Rhizopus nigricans, Macrosporium cladosporioides, Botrytis cinerea u. a.

Dasselbe, was von den Schimmelpilzen gesagt wurde, gilt auch von den Bakterien der Luft, denn die meisten dieser sind lange vorher in Europa bekannt gewesen. Saito hat sie aus der Luft in Tokyo gewonnen und mit bekannten Arten Europas identificiert. Man vergleiche die einschlägige Liste bei Saito.

Eine Bestätigung meiner Ansicht gewinne ich auch aus der Arbeit Hattoris[2], der verschiedene Wasserleitungen in Japan mikrobiologisch untersucht und zahlreiche in Europa und America nachgewiesene Bakterien, Schimmelpilze und Algen auch für Japan festgestellt hat.

Es wäre nicht schwer für hunderte Algen, Flechten, Pilze und Moose die weite Verbreitung auf der Erde nachzuweisen.

In letzter zeit wurde von Miyoshi[3] und Hibino[4] auch die in Europa wohlbekannte Goldalge, Chromophyton Rosanoffii und nach den Angaben

---

[1] Saito K., Untersuchungen über die atmosphärischen Pilzkeime. 1. u. 2. Mitteilung. Journ. of the Science College, Imper. university Tokyo. Japan. Vol. XVIII, article 5. Vol. XXIII article 15.

[2] Hattori, H., Mikrobiologische Untersuchungen über einige japanische Wasserleitungen. The Botanical Magazin, Tokyo, Vol. XI. Article 4.

[3] Miyoshi M., Über das Leuchtwasser und dessen Schutz in Japan. The Botanical Magazin, Tokyo, Vol. 29. 1915. p. 51.

[4] Hibino, S., On Chromulina Rosanoffii etc. Ebenda p. 125.

Todas[1] das bekannte Leuchtmoos Schistostega osmundacea auch für Japan an etwa 70 Orten nachgewiesen.

Nach dieser Sachlage darf es nicht auffallen, dass derselbe Microorganismus, der in Europa das Leuchten des Fleisches verursacht, auch in Japan zu Hause ist.

Bekanntlich wird auch das Leuchten toter Fische und anderer toter Seetiere durch Photobakterien bedingt, aber soweit meine Erfahrungen[2] reichen, niemals durch die Fleischbakterie Bacterium phosphoreum (Cohn) Molisch; sie allein hat sich unter den Leuchtbakterien dem Schlachtviehfleisch angepasst.

In Gegensatz zu der besprochenen Häufigkeit der weiten Verbreittung niederer Organismen steht die relativ beschränkte Verbreitung und der Kosmopolitismus höherer Gewächse, von den Farnkräutern aufwärts.

Hier liegt die Sache wesentlich anders. Ich war bei meinen botanischen Ausflügen in Japan aufs höchste überrascht, zu sehen, wie lange man suchen muss, bis man hier auf Pflanzen stösst, die auch im Mitteleuropa unter annähernd denselben Breitegraden einheimisch sind.

Über die Ursachen dieses Gegensatzes zwischen Mikroorganismen und höheren Pflanzen gedenke ich mich an einem andern Orte zu äussern.

---

[1] Toda Y., Physiological studies on Schistostega osmundacea (Dicks) Mohr. Journ. of the College of Science, Imperial University of Tokyo. 1918. Vol. XL. Art. 5.

[2] Molisch H., Die Leuchtbakterien im Hafen von Triest. Sitzber. d. kais. Akad. d. Wiss. in Wien. Mathem. naturw. Kl. Bd. CXIII Abt. I. 1904. p. 513.

BOTANISCHE BEOBACHTUNGEN IN JAPAN.

Von

Prof. Dr. HANS MOLISCH.

IV. MITTEILUNG.

ÜBER DAS MASSENHAFTE VORKOMMEN VON EIWEISSPINDELN IN EINER VAUCHERIA.

(Mit 1 Tafel)

Als ich eine in einer an ein Reisfeld angrenzenden Wasserrinne wachsende Vaucheria-Art mikroskopisch untersuchte, fielen mir farblose spindelförmige Inhaltskörper auf, die sich von den Chloroplasten dieser Alge durch ihre Grösse und Farblosigkeit scharf unterschieden.

Die Vaucherien besitzen bekanntlich in ihren Zellen, abgesehen von den zahlreichen Chlorophyllkörnern, Fettröpfchen und viele Zellkerne. Ein Inhaltskörper aber, wie ich ihn in den folgenden Zeilen beschreiben werde, war bisher meiner Wissens noch nicht bei dieser Gattung aufgefunden worden.

Entnimmt man dem Rasen ein Büschel Fäden und betrachtet im Wassertropfen bei etwa 500 maliger Vergrösserung, so fallen namentlich in den älteren Teilen, wo die Chlorophyllkörner nicht mehr so dicht liegen wie in den jüngeren, zahlreiche farblose Spindeln auf, die gewöhnlich mit einander zu unregelmässigen, strahligen, mehr oder minder grossen Haufen verbunden erscheinen. Sie erinnern dann etwas an sprossende Hefekolonien oder andere sprossende Pilze. Fig. 1 u. 2.

Derartige Häufchen können aus 5–100 und mehr Spindeln bestehen, die neben und auf einander in Zusammenhang bleiben als ob sie mit einander verklebt wären oder aus einander entspringen würden. Daher sieht man, wenn auf das Deckglas ein sanfter Druck ausgeübt wird, die Häufchen in dem sich bewegenden Zellsaft dahingleiten, während die Chlorophyllkörner in Ruhe verharren.

Die einzelne Spindel hat oft eine Länge von 15 μ und in der Mitte eine Breite von 3.5 μ. Sie lassen sich sehr deutlich machen, wenn man die Vaucheriafäden in concentrierte Schwefelsäure einlegt. Es wird dann abgesehen von der Membran alles zerstört, nur die Spindeln bleiben recht gut erhalten und treten in dem ziemlich homogenen Zellinhalt scharf hervor.

Öffnet man die Zellfäden mit der Schere, so fliesst der Zellinhalt aus und die Spindeln werden mitgerissen. In dem ausrinnenden Saft ändern sie in kurzer Zeit ihre Form, bilden unregelmässige Ballen und sehen wie geronnen aus.

In Kalilauge (10%) bleiben sie erhalten; da die Chlorophyllkörner dabei zerstört werden, wird die Zelle viel durchsichtiger und die Spindeln, die in der unversehrten lebenden Zelle durch die Chlorophyllkörner zum grossen Teile verdeckt werden, treten jetzt klar hervor. Das Reagens setzt den Zellsaft in Bewegung und da die Spindeln sich ebenso wie bei einem ausgeübten Druck mitbewegen, darf man schliessen, dass sie sich nicht im Plasma sondern im Zellsaft befinden.

Nach ihrem ganzen Aussehen und ihrer Ähnlichkeit mit bekannten Eiweisspindeln, war ich geneigt, sie für einen Eiweisskörper zu halten und versuchte daher zunächst die Eiweissreactionen.

Die Xanthoproteinreaction gelang sehr deutlich, die Spindeln werden in conc. Salpetersäure gelb.

Die Raspailsche Reaction gab gleichfalls ein positives Ergebnis, denn sie wurden deutlich rot.

Der Millon'sche Reaction unterworfen, wurden die Spindeln ziegelrot und zwar so deutlich, dass man diese Probe geradezu dazu verwenden kann, um sie in der Zelle trotz der sie verschleiernden Chlorophyllkörner deutlich zu machen.

In Methylgrün-Essigsäure wurden die fraglichen Körper grünblau und coagulieren zu unregelmässigen Ballen. In Delafield's Hämatoxylin bleiben sie schön erhalten und färben sich dunkelviolett.

In absol. Alkohol verquellen sie, scheinen zu verschwinden, aber wenn man sodann mit Osmiumsäure behandelt, kann man die desorganisierten Spindeln an der dunkeln Farbe wieder erkennen. Fig 3.

Alle diese Befunde sprechen grossenteils dafür, dass die Spindeln aus Eiweiss bestehen.

Aber so einfach ist die Sache doch nicht, wie sich aus Folgendem ergibt. Wenn man die Spindeln mit Osmiumsäure (1%) behandelt, so bleiben sie gut erhalten und färben sich schwärzlich blau und zwar so intensiv, dass sie mit diesem Reagens leicht aufgefunden und dauerhaft präpariert werden können. Diese Beobachtung erweckte in mir den Gedanken, dass in den Spindeln vielleicht auch ein Gerbstoff vorhanden sein könnte. Allein die typischen Gerbstoffreationen mit Eisensulfat und Eisenchlorid versagten, nur mit zitronsaurem Eisenammon erhielt ich ein positivs Ergebnis, sie verquellen darin und färben sich nach längerer Zeit blau. Kaliumbichromat-lösung färbt sie gelb. Vor einigen Jahren haben Joachimowitz und ich in dem Dimethylaminobenzaldehyd ein ausgezeichnetes Mittel erkannt, Phloroglucin und Phloroglukotannoide nachzuweisen, da sie sich darin rotviolett färben und Joachimowitz[1] hat die Anwendbarkeit dieses ausgezeichneten Reagens in Pflanzen genauer verfolgt.

Unsere Spindeln geben nun gleichfalls mit diesem Reagens ein deutlich positives Resultat, denn sie färben sich darin nach längerer Zeit unzweifelhaft rötlichviolett.

Das eben erwähnte Verhalten der Spindeln in Osmiumsäure, Kaliumbichromat, Eisenammoncitrat und Dimethylaminobenzaldehyd spricht noch für das Vorhandensein eines andern Körpers, der vielleicht dem Phloroglucin oder den Gerbstoffen nahesteht. Bestimmtes lässt sich darüber vorläufig nicht sagen, doch kann wohl mit ziemlicher Gewissheit geschlossen werden, dass die Hauptmasse der Spindeln aus einem eiweissartigen Körper besteht. Sie seien daher der Kürze halber als Eiweisspindeln bezeichnet.

Will man über die Verteilung der Spindeln in einem Zellfaden ein klares Bild haben, so behandle man die frischen Fäden mit 1%iger Osmiumsäure. Die Spindeln färben sich dann dunkel-bläulichschwarz und geben sich dadurch deutlich zu erkennen. Die Spitze des Fadens erscheint oft homogen schwärzlich, vielleicht weil der fragliche Körper hier in feinerer Verteilung vorliegt, erst in einiger Entfernung von der Spitze tauchen Inseln von Spindeln auf, werden von hier bis zu einer gewissen Entfernung immer dichter und dichter, um dann wieder mehr und mehr abzunehmen und endlich in dem von Chlorophyllkörnern schon ziemlich entblössten alten Teilen ganz oder fast ganz zu fehlen. Fig. 4. In den mittleren Teilen des Fadens sind die Spindeln oft so gedrängt, dass sie einen grossen Teil des Zellraumes erfüllen.

---

[1] Joachimowitz M., Ein neues Reagens auf Phloroglucin, Catechin und ihre Derivate etc. Biochem. Ztschr. Bd. 1917, p. 324.

Über die Species der untersuchten Vaucheria kann ich leider nichts sagen, da sie nicht fructificierte.

Wol aber konnte ich durch Vergleiche festellen, dass die Spindeln sich in vielen aber nicht in allen von mir untersuchten Arten vorfanden, es handelt sich also um einen Inhaltskörper, der entweder nur unter bestimmten Verhältnissen vorkommt oder nur in gewissen Arten gebildet wird. Im Herbste habe ich sie fast regelmässig gefunden, hingegen nicht im Frühjahr. Über ihre physiologische oder ökologische Rolle können wol nur spezielle Versuche entscheiden. Ausser den beschriebenen Spindeln und zeitweise auftretenden Haufen von prismenartigen Kalkoxalat-Kristallen kommen in dem von mir untersuchten Material noch andere Eiweiskristalle vor, die aber eine von den Spindeln ganz abweichende Gestalt haben. Sie weisen rhombenähnliche Flächen auf und sind äusserst selten. In einzelnen Fällen wiesen sie in der Mitte eine deutliche scharfkreisrunde Höhlung auf und ähnelten dann einer viereckigen, in der Mitte vertieften Tuschreibschale. Fig. 5.

Wie diese Höhlung zustande kommt, ist schwer zu sagen, vielleicht wird durch ein proteolytisches Enzym der Kristall von der Mitte zuerst und in sehr regelmässiger Weise abgebaut.

Schliesslich möchte ich auf einen andern sehr merkwürdigen Inhaltskörper hinweisen, den ich in derselben Vaucheria gefunden habe, die Spindeln enthält.

Untersucht man lebende Fäden von der Spitze abwärts gegen die älteren Teile, so sieht man schon in geringer Entferung von der Spitze zwischen den Chloroplasten viele unregelmässige Körnchen von schmutzig bräunlicher Farbe. Fig. 6.

Ihre Grösse schwankt zwischen 0.8 μ–5 μ. Häufig bilden sie krümelige Häufchen bis 10 μ im Durchmesser. Ihre Gestalt ist kreisrund oder unregelmässig rund. Bilden sie mehr oder minder grosse Häufchen, so ist der Umriss meist unregelmässig. Fig. 6. K. Das Einzelkorn zeigt ein helleres Innere und einen dunkeln schmalen Rand. Die Körnchen sind auf den ersten Blick durch Gestalt und Farbe von den grünen Chlorophyllkörnern zu unterscheiden, obwol ihr Inneres bei scharfer Einstellung häufig grünlich erscheint.

In heissem Wasser bleiben sie unverändert oder werden dunkelgrün.

In absolutem Alkohol werden sie dunkelgrün, desgleichen in Äther und Benzin.

In Kalilauge (10%) bleiben sie erhalten, unterscheiden sich aber von den Chlorophyllkörnern durch ihr dunkleres Colorit. In Ammoniak verhalten sich die Körnchen ähnlich.

In Salzsäure nehmen sie eine noch dunklere Farbe an.

Es ist mir leider trotz vielfacher Bemühungen nicht gelungen, irgendwelche charakteristische Reactionen mit den fraglichen Körperchen zu erhalten und daher ist es auch ausserordentlich schwer, ein bestimmtes Urteil über die Körnchen auszusprechen. Wenn ich aber nach all den Eigenschaften, die ich eben angeführt habe, einer Vermutung Raum geben darf, so ist es die, dass die Körnchen vielleicht ein Derivat des Chlorophyllfarbstoffs darstellen.

ERKLÄRUNG DER TAFEL.

Vaucheria sp.

Fig. 1. Teil eines Fadens mit Eiweisspindeln. Verg. etwa 500.

Fig. 2. Dieselben Spindeln, etwa 1760 mal vergrössert.

Fig. 3. Teil eines Fadens nach Behandlung mit Alkohol und Osmiumsäure. s die desorganisierten Spindeln. Vergr. 690.

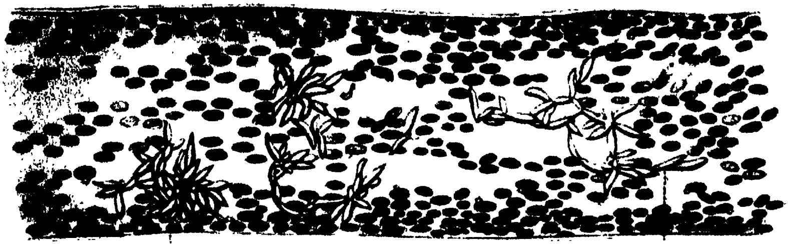
Fig. 4. Verteilung der Eiweisspindeln. Von der Spitze des Fadens nach abwärts zunehmend, dann in den älteren Teilen wieder abnehmend. Vergr. etwa 100.

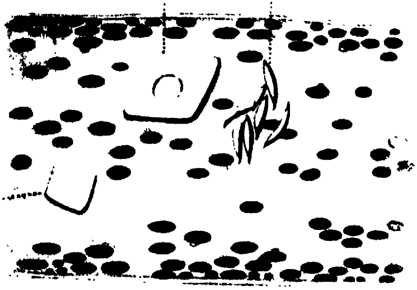
Fig. 5. Teil eines Fadens mit Eiweisskristallen c und Eiweisspindeln s. Vergr. etwa 680.

Fig. 6. Teil eines Fadens mit bräunlichen Körperchen K. Vergr. 690.

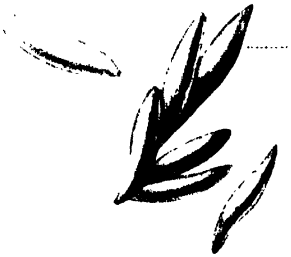
VAUCHERIA
HANS MOLISCH.

1

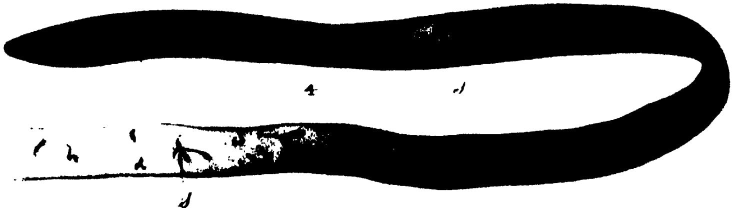




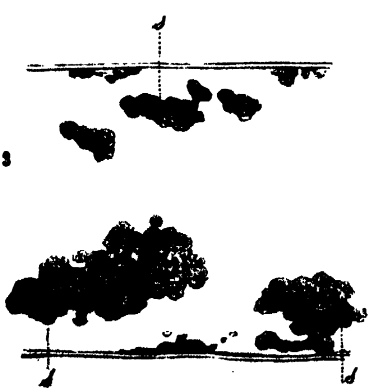
5



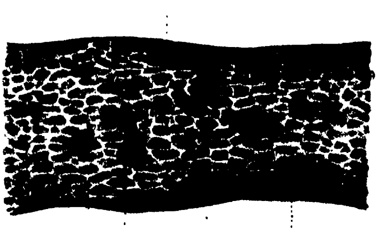
2



4



3



6

(B. Sakuma delin.)

BOTANISCHE BEOBACHTUNGEN IN JAPAN.

VON

PROF. DR. HANS MOLISCH.

V. MITTEILUNG.

MYCOIDEA PARASITICA CUNNINGHAM, EINE PARASITISCHE UND PHYCOPELTIS EPIPHYTON MILLARD, EINE EPIPHYLLE ALGE IN JAPAN.

(MIT 1 TAFEL.)

I. MYCOIDEA.

Beim Studium der Epiphyten in Japan fielen mir auf den Blättern von Camellia, Eurya japonica und Eurya ochnacea eigenartige runde entweder stern-oder scheibenartige Flecke auf, die zunächst den Eindruck einer epiphyllen Flechte machten. Sie wurden einer genauen mikroskopischen Untersuchung unterworfen und dabei ergab sich folgendes.

Makroskopisches. Ich will die Verhältnisse so schildern, wie ich sie bei Camellia beobachtet habe, und bemerke gleich jetzt, dass sie bei Eurya ähnlich liegen. Camellia ist ein in Süd- und Mittel-Japan häufig wildwachsender Baum, der namentlich im Halbschatten des Waldes zusammen mit Aucuba japonica und Litsaea glauca Sieb. an der Zusammensetzung des Unterholzes stellenweise einen gewissen Anteil hat.

Die erwähnten Flecke, die, wie ich gleich vorgreifend bemerken will, durch die Alge Mycoidea gebildet werden, finden sich keineswegs an allen Exemplaren, an solchen aber, die an recht feuchten und schattigen Orten wachsen, kommen die Algenflecke an den alten tiefer gelegenen Blättern nicht selten vor.

Die Alge bildet an der Oberseite des Blattes runde, etwas erhabene Flecke von sehr verschiedener Grösse; von kleinen punktförmigen Inselchen bis zu solchen von dem Durchmesser eines cm. finden sich alle Übergänge. Fig. 1.

Der Organismus wächst ähnlich wie ein strahliges Mycel von einem Zentralpunkt nach allen Seiten und lässt bei genauer Beobachtung schon mit freiem Auge und noch besser mit der Lupe beiläufig radiär verlaufende Stränge erkennen, die sich an der Spitze verbreitern. Fig. 2.

Diese Stränge ercheinen besonders bei jungen Räschen deutlich, bei alten werden sie, da sie hier sehr dicht bei-und über-einander liegen, undeutlich, so dass die Scheibe im Innern mehr gleichmässig aussieht.

Der Rand ist fast stets fein gezackt, strahlig oder gewellt. Fig. 1 u. 3.

Die Farbe der Flecke ist in der Regel, wenn sie alt sind, grau, die der jüngeren an der Peripherie graugrün. Bei älteren Flecken lassen sich mitunter 3 Zonen unterscheiden, eine zentrale gelblich graue, eine mittlere purpurne und eine periphere grauspangrüne Fig. 1.

Nur bei Blättern, die ganz der intensiven Sonne ausgesetzt sind, fand ich die Farbe der Alge orangerot, weil, wie bekannt, durch starkes Licht die Carotinbildung bei den Chroolepideen in hohem Grade gefördert wird. Das Carotin wird dann so massenhaft entwickelt, dass es das Chlorophyll fast ganz oder ganz verschleiert.

Mikroskopisches. Auf Querschnitten durch junge Räschen kann man sofort sehen, dass die Alge die Kutikula abgehoben hat und sich in dem dadurch geschaffenen Raume zwischen Kutikula und Epidermis ausbreitet und ihn vollständig ausfüllt. Mit dem fortschreitenden Wachstum der Alge wird ein immer grösseres Areal der Kutikula und der anhängenden Kutikularschichten emporgehoben und der hiedurch geschaffene Raum wird immer durch die nachwachsenden Algenfäden ausgefüllt. Fig. 5.

Flächenschnitte parallel zur Oberfläche namentlich von jüngeren Stadien lassen erkennen, dass die Alge vielreihige grüne Stränge bildet, die sich zwischen der Kutikula und Epidermis ausbreiten. Bei stärkerer Vergrösserung sieht man in Schnitten, die im Wasser eingebettet sind, den Aufbau dieser Stränge ; sie bestehen aus etwas gestreckten Zellen, die Chlorophyll und häufig auch mehr oder minder reichlich Carotin (Haematochrom) enthalten, wie dies für Algen aus der Gruppe der Chroolepideen charakteristisch ist. Fig. 4.

Von den Algensträngen erheben sich häufig schmale, mehrzellige, spitz zulaufende Haare, die oft reichlich mit Carotin-Tropfen erfüllt sind.

Über die Verteilung und Häufigkeit dieser Haare erhält man eine gute Übersicht, wenn man ein Räschen bei schwacher Vergrösserung (50) in starkem auffallenden Lichte betrachtet.

Die Alge tötet alsbald zunächst die darunter liegenden Epidermiszellen, deren Inhalt sich im Gegensatz zu den gesunden farblosen Zellen tief braun färbt. Es kommt auch nicht selten vor, dass sich vor dem Absterben der Zellen pathologisches Anthokyan bildet und die purpurne Zone, von der oben gesprochen wurde, verdankt ihre Färbung solchem Anthokyan.

Die pathologische Wirkung ertreckt sich dann weiter über die Epidermis hinaus auf die darunter liegenden Pallisadenparenchymzellen, die besonders in den unteren Lagen zu lebhafter Zellteilung parallel zur Oberfläche des Blattes angeregt werden und so die Bildung eines Korkphellogens veranlassen. Die Pflanze sucht sich durch das Korkgewebe von dem Parasiten abzuschliessen. Fig. 5.

In sehr alten Blättern kann die Giftwirkung der Alge noch weiter gehen und das Absterben des Mesophylls unter Braunbärbung bis zur unteren Epidermis des Blattes veranlassen.

Die zerstörende Wirkung der Alge auf das Blattgewebe des Wirtes wird offenbar durch eine von Zelle zu Zelle diffusible Substanz bewirkt, denn die Alge sah ich nur zwischen Kutikula und die Epidermis eindringen, tiefer aber nicht.

In manchen Fällen sah ich unter anderen als eine weitere Folge der Einwirkung der Alge die Bildung von eigenartigen Verdickungen der Wand der unter der Epidermis liegenden Pallisadenzellen eintreten. Diese Verdickungen machen auf den ersten Blick den Eindruck von jenen Balken, wie sie von Sanio zuerst in den Tracheiden von Hippophaë rhamnoides nach Art von Leitersprossen von einer Wand zur andern ausgespannt beschrieben und wie sie später auch an anderen Objekten von verschiedenen Forschern festgestellt worden sind.[1] Allein bei genauerer Betrachtung zeigt sich, dass es sich hier nicht um das Lumen durchsetzende Zellulosebalken sondern um ringartige Verdickungen der Pallisadenzellwand handelt. Fig. 6 r.

---

[1] Die einschlägige Literatur siehe bei Küster E., Pathologische Anatomie, 2 Aufl. 1916, p. 301.

Diese können sich der Grösse der Algeninsel entsprechend auf mehr oder minder grosse Areale erstrecken und bilden dann auf dem Querschnitt des Blattes eine parallel zur Epidermis fortlaufende Linie. Es ist mir nicht bekannt, dass eine solche pathologische Wandringverdickung, hervorgerufen durch einen Parasiten, schon früher beobachtet worden ist. Anstatt eines dicken Ringes können sich noch im oberen Viertel der knapp unter der Epidermis liegenden Pallisadenzelle sehr zahlreiche, ungemein zarte Ringe entwickeln, so dass die Zellhaut in dieser Zone parallel zur Epidermis ringartig gestreift erscheint. Fig. 6 bei b.

Nach der gegebenen Schilderung handelt es sich hier um eine parasitische Alge, die mit der von Cunningham[1] in Indien entdeckten Chroolepidee Mycoidea parasitica Cungh. meiner Meinung nach identisch oder sehr nahe verwandt ist. Der Genannte beobachtete die Mycoidea parasitica an den Blättern des Mango-Baums, von Rhododendron, Farnen, Croton, Thea und Camellia japonica.

Wenn man die Abbildung, die Cunningham von der Alge auf dem Blatt von Camellia japonica gibt, mit der meinigen Fig. 1 vergleicht, so scheinen die Flecke allerdings etwas verschieden zu sein. Das makroskopische Bild ist nicht ganz identisch. Nach Cunningham sind die von Mycoidea parasitica gebildeten Räschen blassgrün bis hell orange. Die Räschen meiner Alge hingegen sind in der Mitte grau und am Rande trüb grün. Orange fand ich sie nur an freistehenden, starker Sonne ausgesezten Bäumen. Soweit ich beobachten konnte, wächst meine Alge zwischen Kuticula und Epidermis, ohne Zellen in die Epidermis oder in das darunterliegende Gewebe zu senden. Cunninghams Alge verhält sich gewöhnlich ebenso, vermag aber mitunter die Zellen auch zwischen den Oberhautzellen tiefer eindringen zu lassen. Dies habe ich bei meiner Mycoidea nie beobachten können.

Die erwähnten Unterschiede lassen es aber nicht berechtigt erscheinen, die in Japan von mir aufgefundene parasitische Alge als eigene von Mycoidea parasitica verschiedene Art aufzufassen. Ich halte daher meine Mycoidea, bevor ich Gelegenheit habe, Tropenmaterial mit dem meinigen zu vergleichen, mit Mycoidea parasitica Cunningh für indentisch.

---

[1] Cunningham D. D., On Mycoidea parasitica, a new Genus of parasitic Algae, and the Part, which it plays in the Formation of certain Lichens. The Transactions of the Linnean Society of London. 2. ser. Vol. 1. Botany. p. 301. London 1880.

Ich fand sie bisher häufig in Sendai, Matsushima, Yamagata, Beppu auf Kyushu und in Fukushima. Um Sendai traf ich sie auch auf den Blättern der verwandten, gleichfalls immergrünen Theacee Eurya japonica und E. ochnacea. Cunningham hat bereits die interessante Beobachtung gemacht, dass Mycoidea sich mit einem Pilz zu einer heteromeren Flechte vereinigen kann und zwar auf den Blättern von Camellia, Nephelium und anderen Pflanzen.

Später hat Ward,[1] zuerst ohne Kenntniss von Cunninghams Entdeckung und Abhandlung, diese von dem letzteren beschriebene Flechtenform der Mycoidea als Strigula complanata Fee fide Rev. T. M. Crombie genau beschrieben. Er hatte Gelegenheit diese Flechte während seiner Tätigkeit auf Ceylon auf den Blättern von Coffea liberica, Ixora, Thea, Memecylon, Eugenia, Anona, Eleagnus, Magnolia, Citrus, Durio, Sideroxylon, Quassia und Michelia zu beobachten und hat ihre Bildung und ihren Aufbau besonders an der letzten Pflanze studiert.

Seine Studie scheint mir deshalb sehr wertvoll, weil sie ebenso wie die Cunninghams so recht deutlich zeigt, wie sich hier vor den Augen des Beobachters der synthetische Aufbau einer Flechte vollzieht und dass sowohl Alge als Pilz für sich in der Natur auftreten können. Daher war es mir besonders interessant, dass ich hier in Japan stets nur die Alge vorfand nie aber einen Flechtenthallus geschweige denn Apothecien beobachten konnte.

Pilzfäden verschiedener Art können auf der Oberfläche des Blattes allerdings gewöhnlich festgestellt werden wie dies in feuchten Ländern überall der Fall ist, doch bildet Mycoidea soweit meine Beobachtungen reichen, in der Umgebung von Sendai keine epiphylle Flechte sondern lebt ohne Pilzsymbionten als parasitische Alge für sich. Ob diese Tatsache mit der Natur der in Japan von mir aufgefundenen Mycoidea zusammenhängt oder mit dem japanischen, von dem der Tropen (Indien, Ceylon) wesentlich verschiedenen Klima oder vielleicht mit dem Fehlen des am Aufban der Strigula-Flechte beteiligten Pilzes, wage ich nicht zu entscheiden.

---

[1] Ward M. H., On the Structure, Development and Life-history of a Tropical Epiphyllus Lichen (Strigula complanata Fee fide Rev. J. M. Crombie). The Transactions of the Linnean Society of London, January 1884 p. 87.

PHYCOPELTIS.

Eine sehr häufige und auf den Blättern der verschiedensten immergrünen Pflanzen verbreitete Alge ist Phycopeltis epiphyton Millardet, Fig. 7 & 8. Sie wurde bisher nur in Europa[1] in den Vogesen und in Baden auf den Blättern der Tanne, des Epheus, der Brombeere und der Leskea gefunden. Sie ist aber, wie ich mich vielfach überzeugt habe, auch in Japan allenthalben zu finden. Ich konnte sie nachweisen auf den Blättern von Aucuba japonica, Pasania sp., Litsea japonica, Camellia japonica, Sarcochilus japonicus, Saccalobium Matsuran, Polypodium falcatum, Lomaria japonica, Lycopodium serratum und auf dem bisher nur in Japan aufgefunfenem reizendem Laubmoose Rhizogonium Dozyanum. Besonders häufig tritt sie an schattigen Orten auf. Die orangeroten Scheiben, die ihre Farbe einer grossen Menge von Carotin verdanken, das das Chlorophyll stark veschleiert, sind schon mit der Lupe dem Geübten ziemlich deutlich sichtbar.

Auf den Blättern der Camellia, Aucuba und Litsea liegen die Scheiben von Phycopeltis manchmal so dicht, dass die Oberseite des Blattes einen orangeroten Ton erhält. Bei dichter Lagerung interferieren die sich berührenden Scheiben und bilden kleine zierliche fächerförmige Decken.

In der Diagnose[1] von Phycopeltis wird angeben: „Thallus scheibenförmig, ungefähr kreisrund, aus einer Zellschicht gebildet, ohne Haare oder Borsten" Dies ist in den meisten Fällen richtig, aber auf den Blättern von Litsea japonica habe ich auf grossen ausgewachsenen Scheiben von Phycopltis auch kurze Haare gesehen. Diese erheben sich gewöhnlich senkrecht zur Oberfläche oft dicht gedrängt und bestehen aus 1–4 kurzen Zellen, von denen die letzte blasen-oder eiförmig angeschwollen erscheint. (Sporangien?) Fig. 9.

Die Haare enthalten auch Carotin und sind in folge dessen orangerot. In meinen Präparaten waren die meisten verschrumpft und zusammengefallen. Ferner heisst es in der Beschreibungen[2] von Phycopeltis: „der Thallus ohne Rhizoiden" in Gegensatz zu Mycoidea. Gewöhnlich beobachtet man tatsächlich von Rhizoiden nichts. Aber ich habe auf dünnen Querschnitten von mit Phycopeltis besetzten Blättern (Litsea japonica) mitunter gesehen, dass vom Mittelpunkte der Scheibe ein oder zwei ganz kurze Rhizoiden in die Epidermis eindringen und dadurch eine Zellwandverdickung der angrenzenden Zellen veranlassen. Fig. 9, r. Der Schnitt muss das Zentrum der Scheibe treffen, wenn man die Rhizoiden sehen will. Sie bleiben ganz kurz und töten die Epidermiszellen nicht.

---

[1] Migula W., Kryptogamenflora von Deutschland, D. Österreich und der Schweiz. Bd. 2., p. 880.

[2] Engler, Prantl, Die natürl. Pflanzenfamilien etc, I. Teil, Abt. 2. p. 108.

Eine solche Phycopeltis-Scheibe liegt dann auf dem Blatte wie ein auf seiner Unterlage befestigter Reisnagel.

Die Auffindung von Phycopeltis in Japan zeigt wieder vom neuen, dass den Mikroorganismen oft eine ungemein weite Verbreitung auf der Erde zukommt und dass sehr viele zum Kosmopolitismus neigen.

Neue Phycopeltis — Arten hat Karsten[1] auf Java auf den Blättern verschiedener Pflanzen gefunden und ihre Entwicklung genauer studiert.

---

[1] Karsten G. Untersuchungen über die Familie der Chroolepideen Annal. Jard. bot. Buitenzorg vol. X. p. 1–66. Pl. I–VI.

ERKLÄRUNG DER TAFEL.

Fig. 1. Blatt von Camellia japonica, besetzt mit Kolonien der parasitischen Alge Mycoidea parasitica. Natürliche Grösse

Fig. 2. Eine junge Kolonie von Mycoidea, sich radiär ausbreitend. Vergr. 20.

Fig. 3. Das Ende eines radiären Astes der Fig. 2 mit Haaren. Vergr. 80.

Fig. 4. Mycoidea parasitica. Das Gewebe eines Thallusastes mit Karotinkugeln in den Zellen, nach Behandlung mit Chloralhydrat. Vergr. 300.

Fig. 5. Blatt von Camellia japonica im Querschnitt mit Mycoidea par. m unter der abgehobenen Kutikula c, h Haar der Alge. Die Epidermiszellen e und das Pallisadenparenchym p braun verfärbt, weil durch die Alge getötet. Darunter korkbildendes Phellogen ph, Vergr. 100.

Fig. 6. Zwei Pallisadenzellen von Camellia japonica, links mit dickem Ring r, rechts mit mehreren ringartigen Zellwandverdickungen r. Vergr. 500.

Fig. 7. Phycopeltis epiphyton, epiphylle Alge, Flächenansicht. a 500-b 300 mal vergrössert.

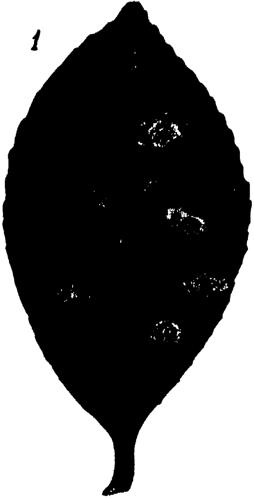
Fig. 8. Dasselbe im senkrechten Durchschnitt. Alge sitzt der Epidermis von Camellia auf. Vergr. 500.

Fig. 9. Dasselbe. In diesem Falle besteht die Alge nicht aus einer Lage von Zellen sondern aus 1-2 Lagen und Haaren h. Bei r ein schwacher Ansatz von 2 Rhizoiden r. Vergr. 500.

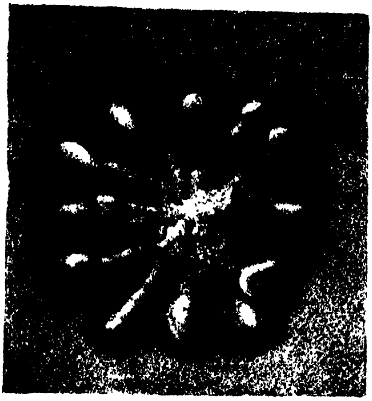
MYCOIDEA u. PHYCOPELTIS

HANS MOLISCH.

1

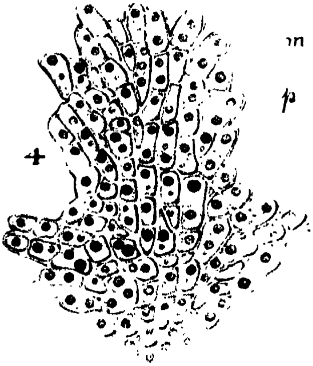


2

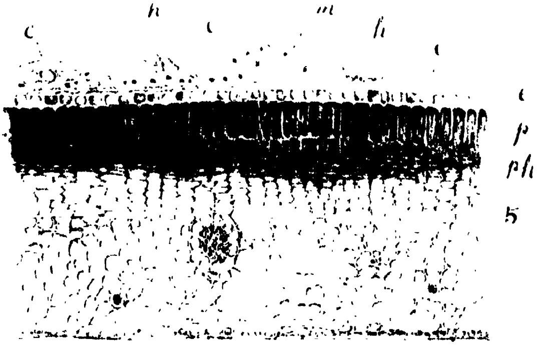


3

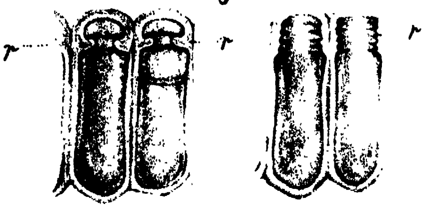
4



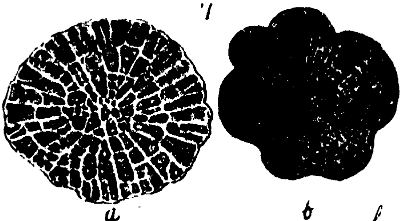
5



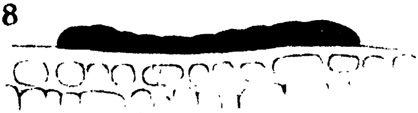
6



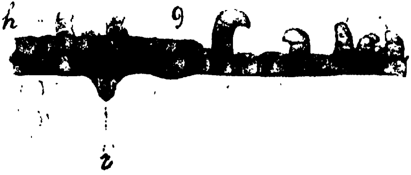
7



8



9



(B. Sakuma delin.)

BOTANISCHE BEOBACHTUNGEN IN JAPAN.

Von

PROF. DR. HANS MOLISCH.

VI. MITTEILUNG.

PSEUDOPLASMODIUM AURANTIACUM N. G. ET N. SP., EINE NEUE ACRASIEE AUS JAPAN.

(MIT 1 TAFEL)

Diagnose: Plasmodium ein unregelmässig verästeltes Netz bildend. Die Äste aus meist spindelförmigen, beiderseits zugespitzten Myxamöben bestehend, welche der Breite nach gewöhnlich in 1–3 Reihen angeordnet sind. Nach und nach kriechen die Amoeben, sich abrundend, auf einen Haufen zusammen von 1/10–5/10 mm Grösse und orangeroter Färbung. Die Färbung der Zellen ist entweder diffus oder wird durch orangerote Körnchen von Carotin bedingt. Keine scharf umschriebenen Fruchtkörper sondern anstatt dieser Haufen (Sori) von runden Zellen. Die Haufen erscheinen dem freien Auge als orangerote Punkte.

Auf faulenden Zostera-Blättern und Bakterienhäuten in Glasschalen mit Meerwasser in Sendai, Japan.

Schon im Winter 1922/23 fand ich auf abgestorbenen Blättern von Zostera marina, die im Meerwasser in einer bedeckten Glasschale halbuntergetaucht lagen rote tropfenartige Punkte von etwa 1 mm in Durchmesser. Später im Juni 1923 sah ich dieselben Punkte auf der Oberfläche von Meerwasser, das ein bedecktes Glasgefäss mit faulen Algen teilweise erfüllte. Auf der Oberfläche dieses Wassers hatte sich eine Haut von Bakterien, zumeist von Thionsäure- und Fäulnisbakterien gebildet, in der sich saprobe Infusorien in grosser Zahl herumtummelten. An einzelnen Stellen dieser Haut sah ich dieselben orangeroten Punkte, die ich bereits von den erwähnten Zostera Blättern her kannte.

Hebt man diese kleinen orangeroten Inseln mit einem Deckglas ab und betrachtet man mikroskopisch, so sieht man folgendes:

Bei schwacher Vergrösserung (50) bietet die Masse ein plasmaartiges Aussehen von unregelmässigem Umriss. In der Mitte erscheint sie geschlossen, an der Peripherie aber geht sie in ein unregelmässiges Netz von Ästen aus, wie man sie in ähnlicher Art bei vielen Plasmodien von Myxomyceten beobachten kann. Fig. 1.

Bei stärkerer Vergrösserung (500) erkennt man dass, die Äste sich aus beiderseits zugespitzten, spindelförmigen Myxamöben zusammensetzen, die der Breite nach in verschiedener Zahl beisammen liegen. Fig. 2. Sind sie einreihig und stellenweise auseinander gerückt, so lassen sie den Zusammenhang durch einen Plasmafaden erkennen. Fig. 3a. Ihre Länge beträgt 8–11 μ. Nach und nach runden sich die spindelförmigen Myxamöben ab, kriechen über einander, sammeln sich in einem kleinen und schliesslich in einem gröseren Haufen an, (Fig. 4) der sich durch orangerote Farbe auszeichnet.

In dieser plasmaartigen Ansammlung kann man insbesondere am Rande, bei Druck auch im Innern die Zusammensetzung aus runden Zellen leicht erkennen. Bei sehr starker Vergrösserung (1050) zeigen viele Zellen 1–3 runde oder etwas gestreckte oder unregelmässige gelbe Körnchen (Fig. 5), die die orangerote Farbe des Myxamöbenhaufens bedingen. Die Körnchen geben die Karotinreaktionen.1) Auffallenderweise findet sich der gelbe Farbstoff in den Spindelzellen nicht in Form von Körnchen sondern in diffuser Verteilung vor.

Zur Bildung bestimmt geformter, scharfumrissener Fruchtkörper kommt es bei unserem Organismus, soweit ich an meinem spärlichem Materiale beobachten konnte, nicht, sondern die Entwicklung findet in der Bildung von grösseren Anhäufungen von sich abrundenden Myxamoeben, die man als Sporen auffassen darf, ihr Ende.

Myxoflagellaten werden nicht gebildet; aus diesem Grunde sowie auf Grund der gegebenen Beschreibung unterliegt es wol keinem Zweifel, dass hier eine neue Gattung der Acrasiaceae vorliegt, die in mehreren Punkten von den bisher bekannten Gattungen und Arten abweicht. Ihre Farbe, beruhend auf der Anwesenheit von Carotin und ihr Vorkommen auf fauligen Objekten des Meerwassers macht sie besonders interessant, umsomehr als bisher Acrasiaceen nur in Europa gefunden wurden und noch nicht in Japan.

---

1) Molisch H., Mikrochemie der Pflanze. 3. Aufl. p. 252, Jena 1923.

2) Engler-Prantl, Die natürl. Pflanzenfamilien. 1. Teil. Abt. 1. Leipzig. 1897, p. 2.

ERKLÄRUNG DER TAFEL.

Pseudoplasmodium aurantiacum n. g. et n. sp.

Fig. 1. Myxamoebenhaufen mit netzartigen, plasmodienartigen Auszweigungen Vgr. 50.

Fig. 2. Ein Teil der netzartigen Auszweigungen, stärker vergrössert, die spindelförmigen Myxamoeben zeigend. Vgr. 500.

Fig. 3. Ein Teil der Fig. 2 noch stärker vergrössert. Die Myxamoeben hängen durch Plasmafäden bei a zusammen. Vgr. 1050.

Fig. 4. Beginnende Anhäufung der Myxamoeben unter gleichzeitiger Abrundung dieser. Vgr. 500.

Fig. 5. Einzelne runde Myxamoeben mit gelben Carotin-Körnchen. Vgr. 1050.

BOTANISCHE BEOBACHTUNGEN IN JAPAN.

VON

PROF. DR. HANS MOLISCH.

VII. MITTEILUNG.

ÜBER WACHSLIEBENDE (CEROPHILE) PILZE.

(MIT 1 TAFEL)

I.

DIE LOKALISATION DES PILZES BEI BAMBUSSEEN, BEDINGT DURCH WACHS.

Es gibt wenige Pflanzen, die eine so ausserordentlich vielseitige Verwendung finden wie die Bambusa- und verwandte Arten. Die in Japan einheimischen und eingeführten Bambusen umfassen 5 Gattungen und 32 Arten und unter diesen gibt es einige, deren Stamm wegen der eigenartigen Farbe oder der fleckenartigen Zeichnungen hochgeschätzt und zur Herstellung verschiedener Gegenstände verwendet werden. Es sind dies die sogenannten „Flecken-oder Buntbambuse." Wir verdanken Kawamura[1] darüber eine wertvolle Abhandlung, in der er unter anderm zeigt, dass sich die genannten Bambuse in 2 Gruppen unterbringen lassen: die einen verdanken ihre auffallende Farbe bestimmten parasitären Pilzen und die anderen verschiedenen Farbstoffen.

Die folgenden Zeilen verfolgen der Zweck zu zeigen, dass manche weit verbreitete Bambusen Flecke an bestimmten Stellen des Rohres aufweisen, die durch einen Pilz von schwarzbrauner Farbe hervorgerufen werden, und dass diese eigenartige Lokalisation des Pilzes durch eine lokale Ausscheidung von Wachs bedingt ist, das dem Pilze zur Nahrung dient.

Bei manchen Bambuseen tritt knapp unterhalb des Knotens ein dunkler Ring auf, der von dem Grün oder Gelb des Internodiums absticht. Dieser Ring kann entweder von Anthokyan herrühren und ist dann tief dunkel, fast schwarzviolett oder er rührt von einem unbekannten sterilen schwärzlichen Pilz her und ist dann schwarz, Solche Pilzringe treten häufig bei Arundinaria Chino, Fig. 4 r, auf.

---

[1] Kawamura S., Über die Flecken-Buntbambuse. The Journal of the College of Science, Imperial University of Tokyo, Japan 1907. Vol. 23., Article 2.

Was ist nun die Ursache dieser eigenartigen, auf den ersten Blick nicht recht verständlichen Lokalisation des Pilzes. Die Ursache ist, wie ich gefunden habe, die Wachskruste, die in Form eines Ringes den Stengel umsäumt. Hier findet der Pilz seine Nahrung und deshalb siedelt er sich gerade an diesem Orte an.

Viele Bambuseen entwickeln an den Knoten deutliche Wachsringe und manche baumartigen Phyllostachys so schöne, dass sie schon von weitem sichtbar sind. Diese Wachsringe wirken geradezu dekorativ und die Schönheit eines Bambuhains mit seinem vom Winde leicht bewegtem Geäste und seinen elastischen Stämmen, die den Künstler wie den Forscher immer wieder gefangen nimmt, wird durch den schneeweissen Wachsreif an den Knoten nur noch gesteigert.

Mikroskopisch betrachtet entpuppt sich der Wachsring als eine dünne Wachsschichte, die senkrecht zur Oberfläche gestreift, doppelbrechend und kristallinisch ist. Sie ist leicht in heissem Alkohol löslich und fällt beim Abkühlen in Form von Nadeln und Häuten wieder aus.

Eine eigenartige Lokalisierung des Wachses fand ich bei Arundinaria Chino. Oberhalb jedes Knotens findet sich (im Herbste) ein strohgelber, das Internodium auf etwa 5 cm fest und ganz umschliesendes Scheidenblatt vor und unter diesem ist die Wachsentwicklung eine so deutliche, dass man nach Wegschaffung dieses Blattes seinen ehemaligen Umriss an dem weissgrauen Wachs gut erkennt. Fig. 4. sb. Knapp unterhalb des Knotens tritt auch bei dieser Art der schon besprochene Wachsring auf. Der erwähnte Wachsüberzug zwischen Scheidenblatt und Stamm verhindert das Eindringen des Regen- und Tauwassers, vielleicht, um die am Grunde des Internodiums fest umschlossene Axillarknospe von der Luft nicht abzusperren und deren Atmung nicht zu hemmen.

Er wird oft von einem schwarzen Pilze besiedelt, so dass die betreffende vom Scheidenblatte bedeckte Stelle nach Entfernung des

Scheidenblattes ganz oder teilweise schwarz ist und den früheren Umriss der Scheide noch deutlich erkennen lässt. Fig. 4. sf.

Der Pilz kann auch über die Scheide hinaus den übrigen Teil des Internodiums in Form von einzelnen Flecken besiedeln und so eine dekorative Wirkung hervorrufen. Fig. 4. a.

Kehren wir nun nach dieser kurzen Abschweifung über Wachs-Überzüge wieder zu den schwärzlichen Stammringen der Knoten von Arundinaria zurück.

Man kann sich leicht überzeugen, dass der dunkle Ring durch das Myzel eines Pilzes gebildet wird. Er lässt sich mit dem Rasiermesser leicht stückweise glatt von der Epidermis abheben. Die Stücke bestehen aus der Wachskruste, auf und in der ein eigenartig gefärbtes, vielfach verzweigtes Myzel wuchert. Es besteht aus verzweigten, zu einem unregelmässigen Netz vereinigten Fäden, die sich aus rundlichen Zellen zusammensetzen und im durchfallenden Lichte unter dem Mikroskope in schmutzig schwärzlich grüner Farbe erscheinen. Oder der Pilz bildet mit seinen Fäden eine braune bis schwarzbraune geschlossene Haut von Pseudoparenchym.

Das Pilzgewebe wird sehr deutlich, wenn man Stücke des schwärzlichen Überzuges in einem Tropfen dest. Wassers einbettet, mit einem Deckglas bedeckt und bis zur Blasenbildung erhitzt. Das die Unterlage bildende Wachs schmilzt, tritt in Form von mehr oder minder grossen Ballen aus dem Bereich des Pilzes in die Umgebung heraus und der nun vom Wachs grossenteils befreite Pilz gestattet dann einen viel deutlicheren Einblick in seinen Bau. Verwendet man anstatt des Wassers absoluten Alkohol, so fällt aus der Lösung bei dem Abkühlen das Wachs entweder in Form von Nädelchen oder Nadeldendriten aus. Es gibt Bambuseen, wo der Stamm vollkommen schwarz ist, wie bei Phyllostachys nigra, Munro und die der schwarzen Farbe wegen sehr geschätzt werden. Hier ist aber nicht ein Pilz die Ursache der schwarzen Farbe sondern ein phlobaphenartiger Körper

---

[1] Bei manchen Arundinaria-Arten hebt sich bei im Freien einseitig beleuchteten Halmen die Epidermis an der Lichtseite spontan ab, ohne dass sie abfällt, und diese abgehobene Oberhaut erweckt den Eindruck, als ob der Stengel an dieser Stelle von einer Wachsschichte bedeckt wäre. Hier erscheint der Stengel grau.
Unterhalb der abgehobenen Epidermis liegen dicke Schichten von Sclerenchymfasern, die aber kein Phellogen bilden und nun die Rolle des Schutzes für das innere Stammgewebe übernehmen.

von dunkelbrauner Farbe, der die Zellen der Epidermis und die darunter liegenden Zellen vollends erfüllt. Im ersten Jahre sind solche Stämme grün, im zweiten werden sie schwarz.

Dass auch Anthokyan sich häufig an der Färbung der Stengel mancher Bambuseen z. B. von Arundinaria-Arten beteiligen kann, wurde bereits früher erwähnt.

II.

Regelmässiges Auftreten wachsliebender Pilze.

a) Acer rufinerve.

Als ich die vielfach innige Beziehung von Wachs und dem Auftreten des schwarzen Pilzes sah, begann ich mich umzusehen, ob sich dieser Zusammenhang nicht auch bei anderen Gewächsen feststellen lässt und solche waren bald gefunden. In erter Linie möchte ich da 3 Pflanzen nennen, die von verschiedenen Gesichtspunkten ein mehrfaches Interesse gewähren und diese sind:

Acer rufinerve, Lindera umbellata und Daphniphyllum macropodum. Betrachten wir zuerst die genannte Ahornart. Es ist eine auffallende Erscheinung, dass die Oberfläche der ein- oder mehrjährigen Zweige regelmässig schwarz erscheint. Wenn der Zweig noch jung ist, ist er von einem Wachsreif bedekt, aber schon im Herbste wird er dunkel, im zweiten bis sechsten Jahre noch dunkler bis er endlich schwarz wird.

Die schwarze Decke kann, mit freiem Auge betrachtet, den Zweig gleichmässig einhüllen oder sie erscheint insel- oder streifenartig und ist durch grüne Partien unterbrochen. Im letzteren Falle, der besonders an kinderarmdicken Stämmen zutage tritt, erhält die Oberfläche des Stammes eine eigene Zeichnung, die an die Haut gewisser Melonen erinnert. Der Japaner bezeichnet daher diesen Baum in sehr treffender Weise als „Melonenhaut-Ahorn oder urihada-kaede."

Die erwähnte Schwarzfärbung rührt nun auffallenderweise von einem ähnlichen Pilz her wie er im Wachs der Bambuseen gefunden wurde und dieser Pilz fehlt, soweit meine darauf konzentrierten Beobachtungen reichen, niemals dem genannten Ahorn. Er gehört einer Torula an.

Die Art, wie er sich auf der Epidermis einnistet, bietet auch anatomisches Interesse. Auf Oberflächenschnitten der Oberhaut sieht man bei schwacher Vergrösserung, der Längsachse parallel, Gruppen zahlreicher dunkler Streifen, zwischen denen mehr oder minder breite spindelförmige Gruppen von mehr gleichmässiger Schwarzfärbung liegen. Fig. 1.

Wie sich bei stärkerer Vergrösserung zeigt, stellen die paralellen Streifen Pilzlinien ss dar, die in entsprechenden, von der äusseren Zellwand der Epidermis gebildeten Rinnen eingebettet sind. Fig. 1. und 2 ss.

Der Querschnitt durch die Epidermis lässt keinen Zweifel darüber, dass das Pilzmycel die Rinnen in der Wand der Oberhautzellen ausfüllt, Fig. 3, s.

Acer rufinerve gehört zu jenen Holzgewächsen, die ihre Epidermis viele Jahre behalten, bevor sie zur Peridermbildung schreiten, und die die Aussenwand der Oberhaut ungemein verdicken.

In folge des Dickenwachstums des Stammes entstehen Spannungen, die zu einem Aufreissen der spröden Kutikula und der Kutikularschichten in der Querrichtung führen.

Die Längsrinnen finden sich schon normal an dem jungen einjährigen Stamm und werden durch die starke Wölbung der Epidermiszellaussenwand verursacht. Durch Spannungen können sie noch breiter und tiefer werden und die Querrinnen verdanken ihre Entstehung hauptsächlich solchen Spannungen in der Epidermis wie sie in Folge des Flächenwachstums der Rinde eintreten.

So wie ein Riss in der Zellwand entsteht, nistet sich alsbald der Pilz darin ein und füllt die gebildete Rinne nach und nach volltändig aus. Daher die schwarzen oder besser gesagt die schwarzbraunen Linien. Fig. 1 und 2. ss.

Bald entstehen aber zwischen den Längsrinnen auch Querrinnen, diese werden im Laufe der Zeit länger und breiter und gleichfalls vom Pilze besiedelt, so dass mehr weniger grose Querstreifen entstehen. Indem von diesen der Pilz schliesslich auf die dazwischen liegenden Felder übergreift, wird ein solches Feld immer dunkler und erscheint schliesslich dem freien Auge ganz schwarz. Fig. 1 und 2, m.

Wird ein etwa daumendicker Stamm mit der Lupe betrachtet, so erscheint seine Oberfläche unregelmässig gefeldert, teils längs- teils quergestreift. Die längsgestreiften Partien machen den Eindruck der jungen eingeschobenen Epidermisteile, die quergestreiften den der alten. Diese Felderung hängt wohl mit dem Flächenwachstum der langlebigen Epidermis zusammen und es liegt die Annahme nahe, dass das Flächenwachstum der Epidermis nicht gleichmässig an allen Punkten des Oberhautmantels erfolgt sondern inselartig, so dass zwischen die schon vorhandenen älteren Epidermisteile neue jüngere eingeschoben werden. Es wäre zu wünschen, dass das Flächenwachstum der ausdauernden Epidermen nicht bloss bei dem eben besprochenen Acer rufinerve sondern im Zusammenhange auch bei anderen Holzgewächsen (Viscum, Lindera, Aucuba, Daphniphyllum etc) untersucht würde.

Niemals habe ich gesehen, dass der Pilz über die Kutikularschichten hinaus in das Lumen der Zelle eingedrungen wäre, er bleibt stets ausserhalb der lebenden Substanz und ist daher als ein Epiphyt zu betrachten.

Der junge Zweig erscheint, wie bereits bemerkt, mit einem weisslich grauen Wachsüberzug überzogen. Dieses Wachs stammt aus der äusseren Epidermiswand, die auch später, wenn der Zweig schon vom Pilze besiedelt ist, ungemein viel Wachs enthält. Wenn man einen Flächenschnitt der Epidermis mit Aethylalkohol unter Deckglas bis zum Sieden erhitzt, so tritt das Wachs in Form kristallinischer Ballen, Kügelchen, Nädelchen oder Dendriten von solchen aus. Das eigenartige Auftreten dieses Pilzes erklärt sich aus seiner Vorliebe für Wachs, das er, wie noch weiter unten gezeigt werden soll, als Nahrung verwertet.

b) Lindera umbellata.

Als ich eines Tages in einem japanischen Restaurant sass und die am Tische befindlichen Zahnstocher betrachtete, fiel mir auf, dass auf einer Seite die obere Hälfte holzfarben, die untere Hälfte aber schwarz war. Die Sache begann mich zu interessieren, ich ging der Sache nach und fand, dass die Zahnstocher von Lindera umbellata und die schwarze Farbe von einem schwarzbraunen Pilz herrührten, dessen Hyphen die Rinde hautartig umspannen. Alle, die ich über die Ursache der dunklen Färbung fragte, dachten nicht an einen Pilz, sondern meinten, dass die schwarze Rindenfarbe der Lindera eigentümlich sei also zum Artcharakter dieses Baumes gehöre.

Der Stamm von Lindera wird aus 3 Gründen zur Herstellung von Zahnstochern herangezogen. 1. ist das Holz hart, 2. hat es einen sehr angenehmen aromatischen Geruch und 3. hat die Rinde die schwarze, durch einen Pilz hervorgerufene Farbe, die hier ähnlich wie bei den Buntbambusen als Ornament wirkt. Schon die einjährigen Zweige sind grossenteils schwarz, stellenweise grün. Durch den Wechsel von Schwarz und Grün kommen schöne wolkenartige Zeichnungen zustande.

Die dunkle Farbe nimmt mit dem Alter zu, wird, wenn der Zweig fingerdick ist, kohlschwarz, später wird er schwärzlich braun. Lindera gehört nämlich auch zu jenen Gehölzen, die ihre Epidermis mehrere Jahre behalten. Diese wird bald mit dem schwärzlichen Pilz mehr oder weniger bedeckt und wird daher schwarz. Wenn aber später schliesslich die Oberhaut abgeworfen und durch Periderm ersetzt wird, so macht die schwarze Farbe einer mehr braunen Platz, weil sich der Pilz auf dem Korke nicht gut entwickelt. Selbst an dem einjährigen, noch grün erscheinenden Zweig ist der Pilz schon vorhanden und bei mikroskopischer Beobachtung sieht man, dass er sich ähnlich wie bei Acer rufinerve, in den parallelen Rinnen der Epidermis einnistet und dadurch die schwarzbraunen Längsstreifen hervorruft. Diese Längsrinnen sind bei Lindera nicht so regelmässig wie bei Acer. Senkrecht zu diesen Längsrinnen entstehen durch Spannungsunterschiede Querspalten, die ebenfalls von dem Pilze bald besetzt werden. Von diesen Längs- und Querrinnen greift der Pilz nach und nach auf die Zwischenfelder über, das Pilznetz wird immer dichter, so dass schliesslich die Oberfläche des Zweiges kohlschwarz wird.

Die Mycelfäden erscheinen bei einer Vergrösserung von etwa 300 braun, septiert und schliessen endlich hautartig zusammen.

Der Pilz, um den es sich hier handelt, ist eine Chaetophoma-Art.[1] In den Wäldern von Sendai habe ich niemals die Zweige von Lindera ohne diesen Pilz gesehen, ich darf sagen, er ist hier eine normale Erscheinung, ob auch anderwärts, bedarf weiterer Beobachtung.

Warum aber siedelt sich der Pilz auf der Oberhaut so regelmässig an? Ein Wachsreif ist allerdings hier nicht zu sehen, aber in der äusseren, ungemein dicken Zellwand ist ebenso wie bei Acer rufinerve sehr viel Wachs eingelagert, wovon man sich in derselben Weise überzeugen kann, wie ich es früher für Acer geschildert habe.

---

[1] Für die gütige Bestimmung dieses und einiger anderer erwähnten Wachs liebenden Pilze bin ich Herrn Prof. M. Shirai zu grossem Danke verbunden.

c) Daphniphyllum macropodum.

Der einjährige Zweig ist zunächst grün, ist aber schon mit einzelnen schwarzen, mit freiem Auge sichtbaren Punkten besetzt. Diese entsprechen einem runden, flach ausgebreitetem Pilzmycel oder dem Fruchtkörper eines solchen.

Der zweijährige Stamm erscheint zum Teil grün, zum Teil schwarz oder in Folge einsetzender Peridermbildung braun.

Die schwarze Farbe kann sich schon auf grössere Areale erstrecken und wenn man mikroskopisch prüft, sieht man, dass die schwarzen Bezirke von einer geschlossenen Haut mit runden Fruchtkörpern des schwarzen Pilzes gebildet werden. In dieser kann man auch die schwarzbraunen Längsstreifen erkennen, die wir schon bei Acer und Lindera beschrieben haben.

Die Unterseite der Blattspreite ist mit einer deutlichen Wachsschichte überzogen und zeigt sehr häufig schwarze Punkte, die demselben Pilze angehören, der sich auf der Stammepidermis vorfindet.

Es ist bezeichnend, dass der Wachsreif dort, wo die Pilzkolonien dichter liegen, verschwindet, ein deutlicher Beweis für die Beziehung zwischen Pilz und Wachs. In Folge des Verschwindens des Wachses wird die betreffende Blattstelle mehr grün, im Gegensatz zu den mit Wachs noch überzogenen Stellen, die weisslich grau bleiben.

Der hier in Frage kommende Pilz ist Leptothyrium Yoshinagai P. Henn. Nach den gemachten Erfahrungen dürfte es nicht schwer fallen, noch andere Beispiele für die innige Beziehungen zwischen gewissen Pilzen und ihrem Vorkommen auf Wachsausscheidungen oder von Wachs reichlich durchsetzten Membranen zu finden.

d) Ein Pilz auf Glaskitt.

Im Anhange sei noch einer Beobachtung gedacht, die ich in einem neuen Gewächshaus des botanischen Garten der Universität Sendai gemacht habe und die mit unserem Thema zusammenhängt. Es fiel mir auf, dass der Kitt, der die Glastafeln in dem Rahmwerk der

Fenster festhält, in den warmen, sehr feucht gehaltenen Abteilungen seine gellbräunliche Farbe einbüsst und an der Oberfläche eine gleichmässig schwarze Farbe anmimmt. Die mikroskopische Untersuchung lehrte, dass die dunkle Färbung von einem Pilze herrührt, dessen Hyphen mehrzellig und braunwandig sind und runde einzellige Sporen erzeugen.

Ich vermutete auch hier eine innige Beziehung zwischen der chemischen Zusammensetzung der Substrates und dem Auftreten des Pilzes und als ich mich nach der Herstellung dieses Glaskittes erkundigte, erfuhr ich, dass er der Hauptsache nach aus Kreide und Öl also unter anderm aus einem Fett hergestellt wird; Offenbar verwertet der Pilz das letztere als Nahrung.

e) Über die Kultur von Pilzen auf Wachs.

Wenn meine geäusserte Ansicht richtig ist, dass das Wachs die Ansiedlung bestimmter Pilze begünstigt und für diese eine Nahrungsquelle darstellt, so muss es auch gelingen, diese Pilze auf Wachs zu kultivieren. Um dies zu prüfen, ging ich in folgender Weise vor.

Als Nährboden verwendete ich Scheiben von Filtrierpapier, die mit gelbem Bienenwachs, wie es käuflich zu erwerben ist, getränkt waren und auf Leitungswasser schwammen.

Zunächst wurden sterile Petrischalen, die mit sterilem Leitungswasser gefüllt waren, vorbereitet. Sodann wurde eine sterile kreisrunde Filtrierpapierscheibe in das heisse geschmolzene Wachs eingetaucht, herausgezogen, abtropfen gelassen und auf das Wasser in die Petrischale eingelegt.

Das Wasser hält den Raum der Petrischale feucht, das Papier von unten nass und bald bildet sich ein feiner Tau auf dem Papier, der dem Pilz das nötige Wasser liefert. Um das Verdampfen des Wassers aus der Schale möglichst zu verhindern, wurden die Petrischalen in grosse Kristallisierschalen mit überklappbaren Deckel gestellt, deren Boden mit einer dünnen Wasserschichte bedeckt war.

Die Impfung wurde in der Weise vorgenommen, dass einige kleine kaum millimetergrosse Fragmente der den Stengel überziehenden Pilzhaut mit einem sterilen Skalpell abgelöst und auf das Wachspapier gebracht wurden. Die Kulturen standen bei Zimmertemperaturen an einem Nordfenster im diffusen Lichte.

a) Versuche mit dem schwarzen Pilz von den Knoten der Arundinaria Chino.

Beginn des Versuchs am 20. Febr. 1924. Geimpft wurden 10 Petrischalen. Schon nach 9 Tagen war Entwicklung unterm Mikroskop zu bemerken. Am 26. März also etwa einen Monat später waren makroskopisch 2 verschiedene Pilze von schwärzlicher Farbe zu unterscheiden, der eine (A) wuchs direkt aus den mit freiem Auge sichtbaren Impfstücken hervor, fruktificierte nicht und glich in seinem Aussehen dem Pilze an den Knoten.

Der andere (B) bildete schwärzlich graue, bis ½ cm grosse kreisrunde Kolonien und produzierte reichlich runde Conidien.

Es ist bemerkenswert, dass, obwohl die Impfmasse verschiedene Keime enthalten haben dürfte, doch nur diese beiden Pilze aufkamen, sogar das fast allgegenwärtige Penicillium, das in Reinkulturen durch seine Aufdringlichkeit so häufig störend eingreift, blieb meistens aus. Das gelbe Wachs führt also zu einer elektiven Kultur, nur cerophile Pilze kommen auf Wachs gut fort und daher behaupten sie hier allein oder fast allein das Feld.

Während wachsliebende Pilze auf gelbem Wachs gut gediehen, war dies beim weissem Wachs nicht der Fall, weil wahrscheinlich beim Bleichprozess Substanzen gebildet oder verwendet werden, die für den Pilz schädlich sind. Auf Butter kamen sie in analog durchgeführten Kulturen gleichfalls gut fort, sie lieben nicht bloss Wachs sondern auch Fett.

b) Versuch mit dem Acer-und Lindera-Pilz.

Auch in den auf gelben Wachs vorgenommenen Kulturen treten je zwei schwärzliche Pilze auf, ein fruktifizierender, Conidien bildender und ein nicht fruchtender; der erstere wahrscheinlich identisch mit dem schon bei Arundinaria erwähnten Pilze B.

Die vorstehenden Versuche haben also mit Sicherheit ergeben, dass es Pilze gibt, die sich auf Bienenwachs entwickeln und gedeihen und da solche Pilze auch in den Wachsüberzügen der genannten Pflanzen vorkommen, so ist der Schluss gestattet, dass sie auch unter natürlichen Verhältnissen das Wachs zu ihrer Nahrung verwerten und sich in diesem sonderbaren Substrat mit Vorliebe ansiedeln.

Zum Schlusse möchte ich noch zweier Pilze Erwähnung tun, die ich auf alten Blättern von Aucuba japonica und Litsea japonica namentlich an feuchten Orten fand und die auf der Oberseite der Blattspreite 1–6 mm breite kohlschwarze runde Flecke bilden.

Die Hyphen des Aucuba-Pilzes zeigen runde sackartige Haftscheiben, die die Pilzfäden an der Epidermis ausgezeichnet befestigen und so die Anpassung des Pilzes an die parasitische Lebensweise deutlich bekunden. Fig. 5. Der Aucubapilz gehört nach Tanaka[1] zur Gattung Asterina, konnte aber mit keiner bekannten Art identificiert werden. Am meisten ähnelt er der Art fimbriata.

Auf Litsea-Blättern finden sich gleichfalls solche kohlschwarze Pilztupfen und ihre Hyphen erzeugen auch Haftscheiben, die aber mehrfach gelappt und für den Litsea-Pilz ungemein charakteristisch sind. Fig. 6. h. Tanaka beschreibt einen auf den Blättern von Cinnamonum pedunculatum vorkommenden Pilz und stellt ihn zur Gattung Dimerosporium. Der von mir auf Litsea gebundene Pilz stimmt mit der Gattung Dimerosporium überein.

Die Kultur dieser Pilze auf Bienenwachs war nicht von Erfolg, sie scheinen demnach von Wachsüberzügen unabhängig zu sein und in der Tat fehlen ja auch solche auf der Oberseite der Blätter dieser Pflanzen.

---

[1] Tanaka E., Vergleich der Gestalt und der Entwicklung der Pilze, die die schwarzen Flecke auf den Blättern von Aucuba japonica, Thea japonica und Cinnamomum pedunculatum bilden. Botanical Magazine. Tokyo 1888, p. 50.

ERKLÄRUNG DER TAFEL.

Fig. 1. Acer rufinerve. Stammoberhaut mit dunkeln parallelen Streifen s. Diese entsprechen Rinnen in der äussern Epidermiszellmembran, die von dem Pilz ausgefüllt sind. Wenn die Epidermis alt wird, ist sie mehr gleichmässig vom Pilze überzogen, so bei dem mittleren Teil m. Vergr. 50.

Fig. 2. Ein Stück von Fig. 1. stärker vergrössert. s parallele Pilzstreifen, m ältere Partie der Epidermis mit dem Pilze überzogen. Vergr. 300.

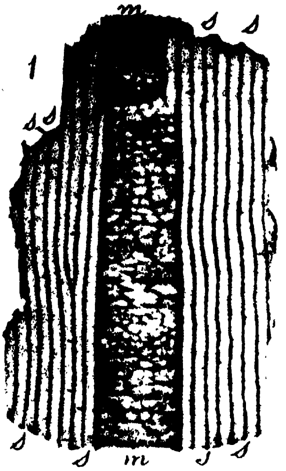
Fig. 3. Querschnitt der Stammepidermis senkrecht zur Längsachse des Stammes von Fig. 2. Die Pilzstreifen s der Fig. 2 entsprechen Rinnen in der Kutikula und den Kutikularschichten mit dem Pilz. Bei m die mehr gleichmässige Bedeckung der Epidermis mit dem Pilz. Vergr. 300.

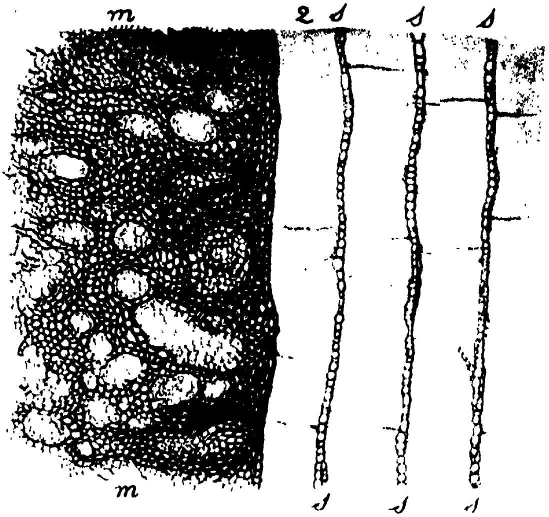
Fig. 4. Drei Halme von Arundinaria Chino. Alle 3 zeigen knapp unterhalb der Knoten schwarze Ringe r, bestehend aus dem Pilz. Er siedelt sich dort an, weil hier Wachs in grössern Mengen gebildet wird. Auch unter dem Scheidenblatt sb wird mehr Wachs produziert und dem entsprechend häuft sich hier der Pilz. Die Umgrenzung des entfernten Scheidenblattes wird durch den Pilz deutlich wiedergegeben. Siehe den mittleren Halm bei sb. Der Pilz kann sich auch unterhalb des Knotenringes ansiedeln z. B. bei a. Nat. Grösse.

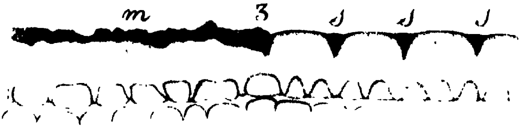
Fig. 5. Aucuba japonica. Blatoberhaut, bedeckt von dem epiphytischen Pilz Asterina mit deutlichen Haftscheiben. Vergr. 140.

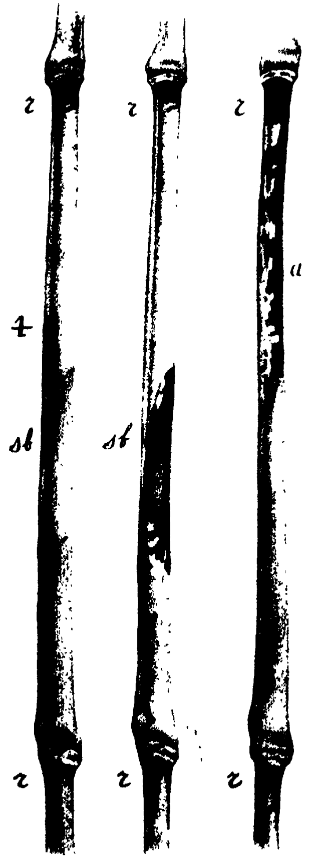
Fig. 6. Litsaea japonica. Blattoberhaut bedeckt von dem epiphytischen Pilz Dimerosporium mit gefingerten Haftscheiben. Vergr. 300.

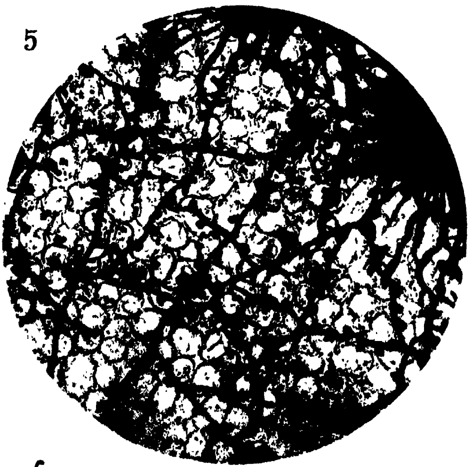
WACHSLIEBENDE PILZE
HANS MOLISCH.

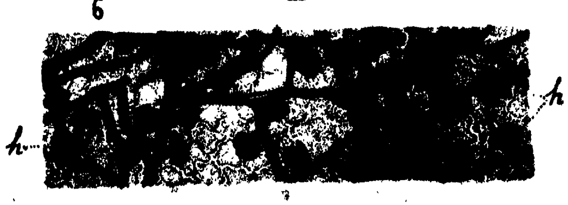












(B. Sakuma delin.)

BOTANISCHE BEOBACHTUNGEN IN JAPAN.

Von

PROF. DR. HANS MOLISCH

VIII. MITTEILUNG.

DIE EISENORGANISMEN IN JAPAN.

(MIT 4 TAFELN UND 2 TEXTFIGUREN.)

INHALTSÜBERSICHT.

I.

Die Eisenbakterien.

1. Einleitung.
2. Allgemeines über das Vorkommen von Eisenbakterien.
3. Über die in Japan aufgefundenen Eisenbakterien.
   - a) Chlamydothrix ochracea.
   - b) „ sideropous
   - c) Clonothrix fusca.
   - d) Cladothrix dichotoma.
   - e) Gallionella ferruginea.
   - f) Toxothrix ferruginea.
   - g) Siderocapsa Treubii.

II.

Andere Eisenorganismen.

1. Algen.
   - a) Oedogonium.
   - b) Characium.
   - c) Closterium.
   - d) Pleurotaenium nodulosum.
   - e) Surirella.
   - f) Cocconeis als Manganorganismus.
2. Flagellaten.
   - a) Anthophysa vegetans.
   - b) Spongomonas intestinum.
   - c) Lagynion.
   - d) Trachelomonas.
   - e) Rhipidodendron splendidum.

3. Tiere: Melicerta, Vorticella, Carchesium.
4. Höhere Pflanzen.
  a) Fontinalis antipyretica.
  b) Trapa natans
  c) Hydrilla verticillata.
  d) Oryza sativa.
Postmortale Eisenspeicherung.
Rückblick.

III.

Über Eisenbacterien, die aus organischen Eisenverbindungen Eisen fällen

a) Harders Versuche.
b) Eigene Versuche
c) Ergebnisse.
d) Schluss

I.

DIE EISENBAKTERIEN.

1. Einleitung.

Da ich mich viele Jahre mit Eisenbakterien beschäftigt und darüber auch eine Monographie[1] geschrieben habe, so war mein Interesse naturgemäss während meines Aufenthaltes in Japan auch auf diese Lebewesen, die in der Natur durch Fällung des in den Gewässern gelösten Eisens eine so bedeutende Rolle spielen, gerichtet.

Kommen Eisenbakterien auch in Japan vor, und wenn dies der Fall ist, wo treten sie gewöhnlich auf, unter welchen Verhältnissen? Sind es dieselben Gattungen und Arten wie in Europa? Spielen sie in der Geologie des Landes eine Rolle und wenn ja, lässt sich dies durch Beobachtungen und Versuche beweisen? Diese und ähnliche Fragen wollte ich behandeln und, wenn möglich, auch beantworten.

Obwohl in Japan auf vielen Gebieten der Botanik eine ungemein rege Tätigkeit herrscht, so hat man sich um diese physiologisch bemerkenswerte Gruppe von Kleinwesen bisher fast gar nicht gekümmert.

Mir ist aus der Literatur nur eine einzige Arbeit bekannt, die sich mit Eisenbakterien in Japan beschäftigt und das ist eine kurze

---

[1] Molisch H., Die Eisenbakterien. Jena 1910.

Mitteilung von Miyoshi[1] über einen Einzelfall des Vorkommens von Eisenbakterien, allerdings über einen recht interessanten. In dem auf dem Abhange des Futatsudake gelegenen Städtchen Ikao befindet sich eine eisenhaltige Thermalquelle, in der eine gänzlich aus Eisenbakterien bestehende Schlammasse vorkommt. Diese überzieht in der aus den Felsspalten hervorsickernden Quelle die Erde und die Steine.

In mikroskopischen Praeparaten des Schlammes sah Miyoshi „eine unzählige Menge von gelblich gefärbten, fadenförmigen Zellen von ungleicher Länge, ca. ½ bis 1μ im Durchmesser, ziemlich geradlinig, auch verschiedentlich gebogen, sowohl einzeln als auch zu Bündeln vereinigt." Die Bakterien scheinen nach Miyoshi der Leptothrix ochracea Ktg. nahezustehen.

2. ALLGEMEINES ÜBER DAS VORKOMMEN VON EISENBAKTERIEN.

Da in der Literatur abgesehen von dieser eben besprochenen Arbeit Miyoshis über Eisenbakterien weiter nichts vorliegt, so könnte man auf die Vermutung kommen, dass diese Bakterien in Japan vielleicht selten und nur an wenigen Orten vertreten sind. Aber das Gegenteil ist der Fall. Auf meinen weiten Reisen, die ich in Süd-, Nord-, Ost- und West-Japan unternommen habe, habe ich mich überzeugt, dass die Eisenbakterien in Japan ungeheuer verbreitet sind und zwar ganz besonders in den berieselten Reisfeldern.

Die Bevölkerung Japans nährt sich hauptsächlich von Fisch und Reis. Wo es die Bodenverhältnisse nur irgendwie erlauben, wird dem Lande jede ebene Fläche abgerungen und Reis darauf gebaut. Ein grosser Teil des Landes besteht seit Jahrhunderten bis auf den heutigen Tag aus Reisfeldern und diese beherbergen in ihrem Inundationswasser allenthalben eine grosse Menge von Eisenbakterien, verschiedene andere Eisenorganismen, Algen und viele höhere Wasserpflanzen wie Lemna minor L. paucicostata L. trisulca, Spirodela polyrhiza, Salvinia, Marsilia quadrifolia, Callitriche verna, C. stagnalis,

---

[1] Miyoshi M., Über das massenhafte Vorkommen von Eisenbakterien in den Thermen von Ikao. The Journal of the College of Science. Imperial University Japan. Vol. X. Part II. Tokyo. 1897, p. 139.

Myriophyllum verticillatum, M. spicatum, Ludwigia, Potamogeton-Arten und andere. Schon mit freiem Auge kann man nicht selten viele Quadratmeter des Wassers im Reisfeld mit einer schleimig rostfarbenen Masse teilweise erfüllt sehen und wenn man sie mikroskopisch untersucht, so findet man, dass sie häufig grossenteils aus Eisenbakterien und gefälltem Eisenoxydhydrat besteht. Es mag dies unter anderm auch damit zusammenhängen, dass der Boden in Japan auf weite Strecken sehr eisenreich ist und deshalb eine ockerige Farbe aufweist. So z. B. in der näheren und weiteren Umgebung von Sendai.

Auch in kleinen Wassergräben und Rinnsälen mit klarem eisenhaltigen Wasser waren oft grosse Mengen von Eisenbakterien zu finden.

Von diesen Orten und von den Reisfeldern sammelte ich überall, wo ich in Japan reiste, so in Süd-, Mittel-und Nordjapan, auf Hokkaido und Sachalin Proben; am genauesten lernte ich allerdings die Verhältnisse in Nordost-Japan um Sendai und seiner Umgebung kennen.

Die mikroskopische Untersuchung liess in den meisten Fällen die typische Eisenbakterie Chlamydothrix ochracea Mig (Leptothrix ochracea Kütz.) erkennen, häufig tritt auch, oft vermengt mit Chlamydothrix ochracea, Gallionella ferruginea auf. Ungemein häufig erscheinen als Epiphyten auf verschiedenen Wasserpflanzen Siderocapsa Treubii, Cladothrix dichotoma, Chlamydothrix sideropous und, jedoch nicht oft, Clonothrix fusca. Besonders im Herbste finden sich epiphytisch lebende Eisenorganismen so häufig, dass die betreffenden Blätter (besonders schön zeigen dies Hydrilla, Hydrocharis, Trapa, Salvinia-Wurzelblätter und Lemnawurzeln) bräunlich oder sogar braun erscheinen.

Auffallend war, dass ich, obwol ich wohl mehrere hunderte Proben darauf hin untersuchte, niemals den in Europa recht häufigen Brunnenfaden, Crenothrix polyspora antraf. Wie ich aus den ausgezeichneten umfassenden Untersuchungen Naumanns[1] über die Eisenorganismen Schwedens ersehe, hat er den Brunnenfaden in den Teichgebiet von Aneboda gleichfalls vermisst.

Um in Japan Eisenbakterien zu erhalten, braucht man keine weiten Ausflüge zu machen, ja ich konnte sie meinen Schülern mitten im

---

[1] Nauman E., Untersuchungen über die Eisenorganismen Schwedens. I. Die Erscheinungen der Sideroplastie in den Gewässern des Teichgebietes Aneboda, K. Svenska Vetenskapsakademiens Handlingar. Bd. 62. Nro 4. Stockholm.

Biologischen Institut der Universität Sendai im Wasserleitungswasser zeigen, als dieses noch aus den Brunnen der Universität zugeleitet wurde.

Fing man in einem hohen Zylinderglas 2–4 Liter dieses Wassers unter dem Hahne der Wasserleitung auf, sellte man das Glas auf ein weisses Blatt Papier und blickte man durch die Wassersäule hindurch auf die weisse Papierfläche, so sah man bräunliche bis schwärzlichbraune Teilchen, die sich allmählig am Boden absetzten und dann mit andern Partikelchen einen gelblichen Belag bildeten. Bei Betrachtung solcher Teilchen unterm Mikroskop erkennt man, dass sie unter anderm aus Eisenoxydhydrat und Eisenbakterien bestehen. Chlamydotherix ochracea und mehr oder minder lange Bruchstücke von Gallionella kommen häufig vor.

In grösseren Mengen lassen sich solche Bakterien gewinnen, wenn das Wasser aus einem Hahne entnommen wird, der wochenlang nicht geöffnet wurde. Das dann daraus entnommene Wasser erscheint durch Eisenrost oft stark braun gefärbt und enthält viele Eisenbakterien.

Die in der Wasserleitung und in der Natur so häufig vorkommende Chlamydothrix ochracea findet sich der Hauptmasse nach in Form ihrer leeren Scheide vor. Keimende und wachsende Individuen kann man sich in vielen Fällen dadurch verschaffen, dass man das Wasser von den Reisfeldern mit etwas organischen Detritus von verwesenden Wasserpflanzen sammelt und mehrere Tage, mit einer Glasplatte bedeckt, stehen lässt. An der Oberfläche des Wassers kann man dann Eisenbakterién und eine oft ganz überraschend mannigfaltige Entwicklung von anderen Eisenorganismen feststellen.

Winogradsky[1] hat bekanntlich ein gutes Verfahren angegeben, nach dem man sich leicht Rohkulturen von Chlamydothrix ochracea verschaffen kann, wenn man in grosse zylindrische Glasgefässe eine Handvoll ausgekochtes Heu legt, frisch gefälltes Eisenoxydhydrat daraufbringt und mit gewöhnlichem Wasser übergiesst. Es entwickeln sich dann nach etwa 2 Wochen braune Flocken und Häute, die neben zahlreichen anderen Bakterien Infusorien, Amoeben und Flagellaten, viel von der genannten Eisenbakterie enthalten. Solche Versuche ergaben mir in Prag und Wien ausgezeichnete Resultate. In Sendai aber waren die Ergebnisse nicht befriedigend; es traten bei dem erwähnten Winogradskyschen Versuch entweder keine oder nur sehr wenige Eisenbakterien auf, vielleicht weil das Wasser hiezu nicht besonders geeignet war oder weil das Heu aus anderen Pflanzen bestand als in Europa. Es gelang mir aber sehr schöne Rohkulturen dadurch zu erhalten, dass ich Flusswasser anstatt mit Heu mit Brocken der humificierten basalen Teile von Laubmoosrasen (Bartramia etc.) und etwas Eisenammoncitrat versetzte. Nach 2-3 Wochen war dann besonders an der Wasseroberfläche und knapp darunter eine braune flockige Haut zu beobachten, die grossenteils aus den Fäden der Chlamydothrix ochracea bestand,

---

[1] Winogradsky, S., Über Eisenbakterien. Botan. Ztg. 1888. S. 263.

3) ÜBER DIE IN JAPAN AUFGEFUNDENEN EISENBAKTERIEN.

a) Chlamydothrix ochracea (Kütz.) Mig.=Leptothrix ochracea Kütz. Siehe Tafel I. 3.

Diese typische Ockerbakterie herrscht in den auf den Reisfeldern stehenden Wässern unter den fädigen Eisenbakterien vor und es scheint mir bemerkenswert, dass sie gerade im Wasser der Reisfeldern üppig gedeiht, die stark gedüngt werden. Die Feldkultur wird ja in Japan seit Jahrunderten mit einer Sorgfalt und Sachkenntniss betrieben, dass sie mehr den Eindruck einer Gartenkultur macht. Dabei wird überaus kräftig gedüngt und dies gilt auch von den Reisfeldern. Daher muss das auf dem Reisfelde stagnierende Inundationswasser sicherlich eine nicht unbeträchtliche Menge organischer Substanz enthalten, die auch der genannten Ockerbakterie zugute kommen und ihr Gedeihen fördern mag. Dies steht im Einklang mit meinen vieljährigen Erfahrungen, dass Chlamydothrix ohne organische Substanz nicht ausgiebig wächst. Ob dies für alle andern Eisenbakterien auch gilt, will ich nicht behaupten, doch ist bisher die rein autotrophe Ernährung mit Eisen noch von keiner Eisenbakterie einwandfrei bewiesen. Lieske[1] gibt an, dass es ihm gelungen sei, Chlamydothrix ochracea in rein anorganischer Nährlösung zwar nicht mit Eisen wol aber mit Manganbikarbonat zu ziehen. Die Entwicklung von Spirophyllum ferrugineum in rein mineralischer Lösung wurde von dem genannten Autor[1] gleichfalls behauptet, allein dies bedarf einer Nachuntersuchung, weil inzwischen von Cholodny (siehe Seite 143) gezeigt wurde, dass Spirophyllum nichts anderes als Galionella ist und diese wieder nicht anderes als das Eisenskelet eines vielleicht flagellatenartigen Organismus darstellt, der auf der Spitze der zopfartig gewundenen strukturlosen Fäden aufsitzt und diese abscheidet.

---

[1] Lieske R., Zur Ernährungsphysiologie der Eisenbakterien. Zentralbl. f. Bakteriologie etc. 2. Abt. 49. Bd. p. 422.

Mit meinen Erfahrungen über die Bedeutung organischer Stoffe für gutes Gedeihen von fädigen und anderen Eisenbakterien in Einklang stehen auch die Beobachtungen Naumanns über Cladothrix, Chlamydothrix und Sideroderma in Schweden. „Anreicherungsversuche in dem Laboratorium haben nämlich gezeigt, dass diese Formen stets dann zu luxurieren beginnen, wenn ein reichlicher Gehalt an faulenden Pflanzenstoffen dem Wasser beigemischt wird. Es ist ja dies auch ein Fall, der in den Eisensümpfen unter natürlichen Bedingungen sehr oft vorliegt."[2]

Im Herbste findet man in dem ockrigen Massen meist nur die leeren vererzten Scheiden, doch sind namentlich nach Behandlung mit verdünnter Salzsäure in einzelnen Scheiden die Zellen deutlich zu erkennen. In Anreicherungskulturen lassen sich jedoch Keimlinge oft in grosser Zahl auffinden.

b) Chlamydothrix sideropous Molisch.

Diese von mir[3] seinerzeit beschriebene leicht erkennbare Eisenbakterie ist weit verbreitet, spielt aber nicht jene hervorragende Rolle in der Natur wie Chl. ochracea. Ch. sideropous findet sich epiphytich oder auf dem Spiegel stagnierender Wässer vor, bildet eine von eingelagertem Eisenoxyd braune, meist runde Scheibe, von deren Mittelpunkt der Faden ausstrahlt und ins Wasser taucht. Fig. 7, ab Tafel II. Liegen die Haftscheiben dicht neben einander, so können sie zu grösseren braunen Häuten zusammenfliessen, wie dies deutlich durch die Fig. 7 c auf Tafel II veranschaulicht wird. In Laboratoriumskulturen treten sie am Wasserspiegel oft massenhaft auf.

---

[1] Lieske R., Beiträge zur Kenntnis der Physiologie von Spirophyllum ferrugineum Ellis, einem typischen Eisenbakterium. Pringsheims Jahrb. f. wiss. Botanik. 49. B. p. 91.

[2] Naumann E., l. c. p. 36.

[3] Molisch H., l. c. p. 14.

c) Clonothrix fusca Schorler.

Auf Wasserpflanzen und Pflanzendetritus der Reisfelder und Tümpel vorkommend aber nicht in bedeutender Menge. In besonders schöner Entwicklung habe ich diese Bakterie auf den Wurzelhaaren von Hydrocharis asiatica gesehen, wo jeder Faden oder jedes Fadenbündel auf einer klumpigen Eisenoxydmasse aufsass. Fig. 4 auf Tafel I.

d) Cladothrix dichotoma Cohn.

Ein ungemein weit verbreiteter Organismus in Tümpeln, Rinnsälen und Reisfeldern, zumal wenn diese Wässer mit organischen Substanzen verunreinigt sind. Gewöhnlich ist aber die Einlagergung von Eisen so schwach, dass die Fäden farblos erscheinen und ihr Eisengehalt erst durch die chemische Reaction erkannt wird. Nur in eisenhältigen Gewässern und in Rohkulturen des Laboratotiums wird die Scheide zumal an der Basis des Fadens rostbraun.

Ähnliches hat auch Naumann[1] im Teichgebiet Anebodas in Schweden beobachtet.

e) Gallionella ferruginea Ehrenberg.

Das, was man bisher als Gallionella ferruginea (einschlieslich Spirophyllum) bezeichnet hat, wurde fast allgemein als eine Eisenbakterie aufgefasst. Aber es war mir stets auffallend, dass diesem Organismus d. h. dem zopfartig gewundenen eisenreichen Doppelfaden die Qualitäten einer Zelle oder eines Zellfadens abgehen. Niemals konnte ich mich von der Anwesenheit eines Protoplasma, eines Lumens, einer Wand oder an einander gereihten Zellen überzeugen, während bei anderen Eisenbakterien wie Chlamydothrix ochracea, Clonothrix fusca und Cladothrix dichotoma die Zellen jederzeit entweder direkt oder nach Einwirkung verdünnter Salzsäure oder Farbstofflösungen zu sehen sind. Auch die von Ellis aufgestellte Gattung Spirophyllum lässt die erwähnten Eigenschaften einer Zelle vermissen und dieses sonderbare Verhalten wurde mir noch befremdender als ich mich davon überzeugte, dass Gallionella und Spirophyllum sich in Salzsäure restlos lösen[1] Dieses Rätsel wurde nun vor kurzem durch eine kleine, aber ebenso durch gute Kritik wie scharfe Beobachtung gleich ausgezeichnete Arbeit gelöst, die wir N. Cholodny[2] verdanken. Dem genannten Forscher gelang es nachzuweisen, dass die von verschiedenen Verfassern unter den Bezeichnungen Gallionella, Spirophyllum und dergleichen bestehenden Produkte nichts anderes sind als aus Eisenoxydverbindungen bestehende Bildungen der Lebenstätigkeit von Mikroorganismen. Oder mit andern Worten: Sowie manche Flagellaten z. B. Anthophysa vegetans eisenreiche, einem verzweigten Sträuchlein ähnliche Skeletbildungen absondern, auf deren Astspitzen die Kolonien sitzen, so liegen auch auf der Spitze der Doppelfäden von Galionella wie auf einer zweizinkigen Gabel je eine oder je 2 Zellen auf. Fig. 1.

---

[1] Naumann E., l. c. p. 36.

Die Form und Grösse dieser Zellen sind nach Cholodny nicht überall gleich sondern sind, wenn einzeln vorhanden, grösser (1.2 × 0.5 μ) und haben eine nieren-oder bohnenförmige Gestalt. Die konkave Seite der Zelle ist stets dem Stamme zugekehrt, die andere von diesem abgewendet. Wenn zu zweien vorhanden, sind sie viel kleiner, etwa 2 mal kleiner und sehen Kokken oder Kokkenbacillen ähnlich. Diese entstehen nach dem genannten Autor aus den grösseren Zellen durch Teilung und jede von den Tochterzellen scheidet einen neuen selbständigen Ast ab. Bezüglich weiterer Einzelheiten sei auf das Original verwiesen.

Diese Gallionella, die sich bei weiterer Untersuchung vielleicht als ein Flagellat entpuppen wird, ist ein in Japan ungemein verbreiteter Organismus. Ich habe seine zopfartigen Bildungen in den ockerigen Ablagerungen der Reisfelder, vieler eisenreicher Wasserrinnen und Tümpel häufig aufgefunden. Die ockerigen Massen der Reisfelder bestehen oft grossenteils aus diesen Skeleten, nicht selten vermischt mit Chlamydothrix ochracea. Wie in Europa habe ich mich auch in Japan von der Formenmannigfaltigkeit dieser Gallionellabildungen überzeugt und diese grosse Verschiedenheit wird auch von Naumann[3] betont. Ob diese alle ein und derselben Art angehören, wird man

---

[1] Molisch H., Die Eisenbakterien. p. 24. Jena 1910.

[2] Cholodny N, Zur Morphologie der Eisenbakterien Galionella und Spirophyllum. Ber. d. Deutsch. Botan. Ges. Bd. 42 1924, p. 35.

[3] Naumann E. l. c. p. 37–41.

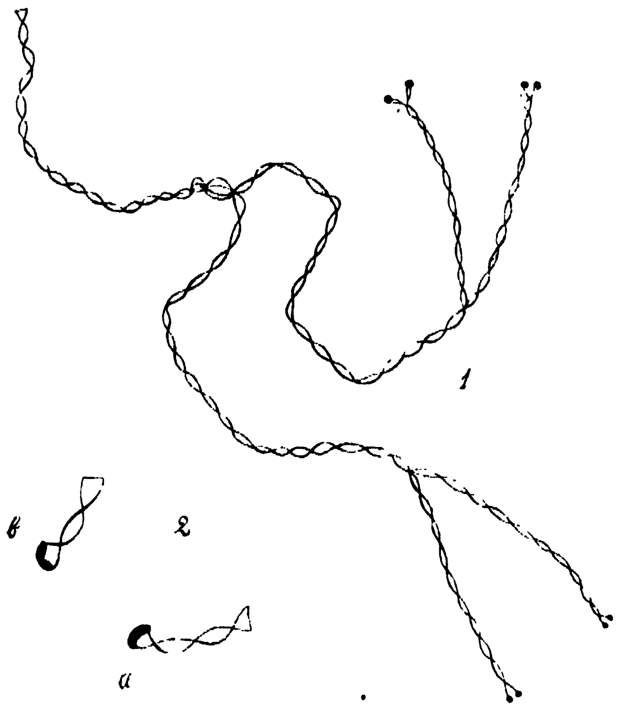


Abbildung I. Gallionella ferruginea.

1. Dichotom verzweigtes Eisenskelet mit auf der Spitze aufsitzenden Zellen, die das Skelet abscheiden. Vergr. 1000.
2. Ende des Skelets mit den Zellen, 2400 mal vergrössert, a eine alte nierenförmige Zelle, b zwei junge, eben durch Teilung entstandene Zellen, nach Cholodny.

erst dann entscheiden können, wenn man die dazu gehörigen lebenden Zellen wird vergleichend beobachten können.

Die Vertreter der Naumannschen Mycogalliomella habe ich in Japan nicht gefunden, doch will ich damit keineswegs behaupten, das sie in Japan überhaupt nicht vorkommen.

f) Toxothrix ferruginea n. g.

Im Folgenden gebe ich die Beschreibnng eines neuen Organismus, den ich um Sendai einige Male aufgefunden und dem ich wegen seiner im Bogen verlaufenden Fäden provisorisch den Namen Toxothrix ferruginea (τόξον der Bogen, ςίξ das Haar) gebe.

Diagnose: Der einzelne Faden strahlt oft in ein Bündel von meist bogenförmig verlaufenden feinen Fäden aus, die sich wieder zu einem gemeinsamen Fadenbündel vereinigen können. Häufig splittern sie nochmals auf und laufen dann wieder in einem Faden zusammen. Solche Gestalten erinnern an ein Brustkorbgerippe. Die Dicke des Mutterfadens schwankt zwischen 0.8–10 μ, die der Tochterfäden zwischen 0.4–1.5 μ und die Länge der verzweigten Fadenstücke zwischen 100–300 μ und mehr. Fig. 1–5 auf Tafel II.

Die Fäden sind ockerartig braun, weil mit Eisenoxyd incrustiert, und stellen massige ockerige Niederschläge in eisenhaltigen Wässern der Reisfelder dar. Die Qualitäten der Zelle an den Fäden habe ich hier ebenso vermisst wie bei den Fäden der Gallionella. Est ist mir daher sehr wahrscheinlich, dass sich die Sache hier ähnlich verhält wie bei Gallionella und dass das, was ich bisher sah, vielleicht nur die von lebenden, an der Spitze der Fäden sitzenden Zellen abgeschiedenen Bildungen sind, vielleicht verursacht von bestimmten Bakterien oder Flagellaten. Sowie bei Anthophysa die Kolonien sich schon bei geringen Störungen vom Skelete ablösen, so mag dies auch hier stattfinden und dies dürfte der Grund sein, warum ich den eigentlichen Bildner dieser eigenartigen fädigen Skelete nicht zu Gesichte bekam.

Das Ganze bedarf noch weiterer Untersuchungen und wenn ich hier doch schon darauf zu sprechen komme, so hat dies lediglich den Zweck, die Aufmerksamkeit darauf zu lenken, da es sich augenscheinlich nicht um eine rein anorganische Bildung sondern um eine biologische handelt, die sich von anderen fädigen Eisenorganismen auf den ersten Blick unterscheidet.

g) Siderocapa Treubii Molisch.

Gehört nicht nur in Europa sondern auch in Japan zu den häufigsten Eisenbakterien. Sie findet sich als Epiphyt auf der Epidermis der verschiedensten Wasserpflanzen, besonders schön auf der Mitterippe der Oberseite der Blätter von Blyxa caulescens Maxim. Siderocapsa bildet kleinere und grössere Inseln von tief brauner Farbe und lässt in den hellen zentralen Höfen die schwer färbbaren Bakterien gewöhnlich in der Ein- bis Dreizahl bei starken Vergrösserungen erkennen. Fig. 6. abcd auf Tafel II.

Auf S. 49 des zitierten Werkes beschreibt Naumann ellipsoidische Eisenflecke auf der Epidermis von Potamogeton natans, die er einer neuen Siderocapsa, der S. monoica zuschreibt. Die von diesen Rostflecken entworfene Zeichnung (Fig. 30) hat mich sehr an die Eisen- und Manganflecke verschiedener Wasserflanzen erinnert, die unter dem Einflusse starken Sonnenlichtes durch die grüne Pflanze und nicht durch Bakterien erzeugt werden. Siehe 154. Die durch Siderocapsa gebildeten Rostflecke können bei oberflächlicher Betrachtung leicht mit den Haftscheiben der Chlamydothrix sideropous und denen einiger Algen (Oedogonium-Arten etc.) leicht verwechselt werden, worauf hier in Übereinstimmung mit Naumann[1] ganz besonders aufmerksam gemacht werden soll.

h) Chlamydothrix thermalis n. sp.

In eisenhältigen Thermen von Japan fand ich nicht selten eine ungemein feinfädige Bakterie, die der Chlamydothrix hyalina ähnelt, sich aber von dieser durch noch feinere Fäden, ihr Speicherungsvermögen für Eisen und ihre Vorliebe für Thermen unterscheidet. Ich nenne sie daher Chlamydothrix thermalis.

Diagnose: Feine, unverzweigte Fäden von 0.4–0.5 Dicke. Scheide und Zellen kaum erkennbar. Scheiden können Eisenoxyd speichern und nehmen dann eine bräunliche Farbe an. Dichte Häute und Bündel bildend.

Gefunden in verschiedenen Thermen Japans unter anderen in der heissen Quelle von Sumaki bei Shiwobara und in Narugo. (Temp. 51)

II.

ANDERE EISENORGANISMEN.

Die bisher angeführten Eisenbakterien spielen bei der Praecipitation des Eisens in der Natur zweifellos eine wichtige Rolle, die einen mehr die andern weniger. Ganz hervorragend beteiligt sind hiebei in Japan Chlamydothrix ochracea, Galionella ferruginea in der oben gegebenen Fassung und Siderocapsa Treubii.

---

[1] Vgl auch Naumann E., Untersuchungen aus dem Öresund X. Über eine Lagynion-Siderocapsa-artige Structur in marinem Aufwuchs vom Öresund, p. 9. Lunds Universitets Årsskrift N. F. Avd. 2. Bd. 19, Nro 5. Kungl. Fysiografiska Sällskapets Handlingar. N. F. Bd. 34. Nro 5.

Aber neben diesen an der biogenen Festlegung des Eisens beteiligten Eisenbakterien kommen noch viele andere Eisenorganismen in Betracht, deren Mitwirkung mit Rücksicht auf ihre Häufigkeit nicht unterschätzt werden darf. Ich habe schon in meiner Monographie der Eisenbakterien darauf aufmerksam gemacht, verweise, um Wiederholungen zu vermeiden, auf die dort zitierte Literatur (p. 53) und will hier nur folgendes kurz hervorheben.

Manche Conferven pflegen sich häufig mit Röhren und Gürteln von Eisenoxydhydrat zu umgeben und wurden von Kützing in der Gattung Psichohormium vereinigt. Klebs hat den Eisengehalt der Membran verschiedener Closterium-Arten erkannt und Molisch und Naumann[1] haben diese Befunde erweitert. Ich komme auf manche dieser Ergebnisse in den folgenden für Japan geltenden Ausführungen noch zurück.

1. ALGEN.

a) Oedogonium.

Dass verschiedene Oedogonium-Arten eine besondere Anziehungskraft für Eisen bekunden, wurde von mir[2] schon vor 33 Jahren erkannt. Die epiphytischen Oedogonien entwickeln eine mehr oder minder grosse flache Haftscheibe, die in Folge der Incrustation mit Eisenoxydhydrat rostbraun erscheint. Wenn sich der Oedogonium-Faden von der Haftscheibe abgelöst hat, so ist diese bei oberflächlicher Betrachtung mit Siderocapsa-Rostflecken oder den Haftscheiben von Chlamydothrix sideropous leicht zu verwecheln, worauf ich besonders aufmerktam machen möchte.

Auf die Haftscheibe kann die Fällung der Eisens beschränkt bleiben. Wenn der Keimling noch jung und einzellig ist, lässt er ein farbloses, einfach, zweifach oder mehrfach gelapptes Rhizoid erkennen. An diesem kann die Vererzung an der Grenze zwischen der grünen und der farblosen Zone kragenartig einsetzen, Fig. 5 a, c, Tafel III. oder die Eisenabscheidung beginnt an der Spitze des Rhizoids oder längs seiner ganzen Oberfläche, sich von hier immer mehr und mehr ausbreitend. In eisenreichen Wässern kommt es aber auch vor, dass die Oedogonium-Fäden sich auf kürzere oder längere Strecken mit einer deutlichen Kruste von Eisenoxyd bedecken, ähnlich wie manche Conferva-Arten. Oedogonium sitzt auf den Wasserpflanzen eisenreicher Tümpel, Wassergräben und Reisfelder häufig auf und zeigt dann die erwähnten Incrustationen.

---

[1] Naumann E., l. c. p. 22–23.

[2] Molisch H., Die Pflanze in ihren Beziehungen zum Eisen. Jena 1892, p. 12.

b) Characium.

Viele Arten haben die Eigentümlichkeit, sich mit einer Haftscheibe ähnlich wie Oedogonium-Keimlinge festzusetzen.

Diese lagert so viel Eisenoxydhydrat ein, dass sie eine tief braune Farbe annimmt. Man kann alle Entwicklungsstadien der Characien leicht finden, wenn man eisenhältige Wässer von den Reisfeldern mit etwas Wasserpflanzendetritus in einem Glase stehen lässt. Auf der Wasserhaut sitzen dann eine Unzahl Keimlinge, die sich von ihrer braunen Haftscheibe erheben. Fig. 4, Tafel III. Nach Entfernung der Zelle bietet die zurückbleibende Erzscheibe ein Bild dar, das lebhaft an Lagynion und Siderocapsa erinnert, bei einiger Sorgfalt aber unschwer zu unterscheiden ist.

c) Closterium.

Wenn Arten dieser Gattung ein gewisses Alter erreichen, so lagern sie, wie bekannt, häufig in ihrer Membran Eisen so reichlich ein, dass diese rostbraun erscheint. Geringere Eiseneinlagerung lässt sich durch die Eisenreactionen prompt nachweisen.

Bekanntlich besteht die Zellhaut der Desmidiaceen aus zwei Hälften, die in der Mitte der Zelle auf eine ganz kurze Strecke über einander gestülpt sind. Die eine Hälfte ist immer älter als die andere. Welche von beiden die ältere ist, kann oft leicht festgestellt werden, wenn man die Zelle der Eisenreaction unterwirft. Lässt man gelbes Blutlaugensalz und Salzsäure einwirken, so färbt sich die alte Hälfte, weil sie mehr Eisen enthält, tiefblau, die junge aber nicht oder viel weniger. Man kann auf diese Weise durch eine chemische Reaction Jung und Alt unterscheiden. Fig. 6, a, Tafel III.

d) Pleurotaenium nodulosum.

Diese Alge ist um Sendai in Tümpeln nicht selten. Sie besitzt in der Mitte der Zelle eine Einschnürung, in der ein aus Eisenoxyd bestehender brauner Ring die Zelle wie ein Armband das Handgelenk umfasst.1) Fig 1 a und b Tafel III. Ich weiss aus Erfahrung, dass eine solche äusserst zierliche, streng lokale Eisenablagerung noch bei einigen anderen Desmidiacen z. B. bei der auf Tafel III. Fig. 2. abgebildeten vorkommt, doch ich will darauf nicht näher eingehen, weil ein Schüler von mir, Herr Dr. K. Höfler es unternommen hat, diesen Gegenstand genauer zu untersuchen. Auch die Membran alter Zellen kann Eisen speichern und dann kann man ebenso wie bei Closterium die ältere von der jüngeren Membranhälfte durch den Grad der Eisenreaction leicht unterscheiden.

e) Surirella.

Diatomeen zeigen im allgemeinen keine Neigung zum Festhalten des Eisens. Als Eisenfäller hat Naumann in Schweden die Eunotia impressa v. angusta Grun. erkannt. In Japan habe ich diese nach Art einer Cocconeis epiphytisch festsitzende Kieselalge nicht gesehen, hingegen habe ich, allerdings nur einmal, eine ockerige Masse in Wasser eines Reisfeldes am Fusse des Berges Otakomori bei Matsushima gefunden, die zum grossen Teil aus einer Surirella-Art vermengt mit Eisenoxyd bestand.

Diese durchschnittlich 48–60 μ lange und etwa 16 μ breite Alge war von einer bis zu 3 μ dicken Lage von oft körnigem Eisenoxyd bedeckt. Fig. 3, Tafel III. Häufig löst sich die Eisenhülle als geschlossene Decke von der Kieselhaut der Diatomee scharf ab. Die Blaufärbung nach Behandlung mit Ferrocyankalium erstrekt sich nicht bloss auf die Eisenauflagerung sondern nicht selten auch auf die Kieselmembran, doch ist der hier vorhandene Eisengehalt im Vergleich zu der Aussenhülle gering.

---

1) Der Eisenring bildet sich nach und nach an der Aussenseite der Zellhaut aus. Wie ist eine solche scharfe Localisation des Metalls an der äusseren Oberfläche der Membran möglich, wenn der Ring vom lebenden Plasma durch die Wand getrennt ist? Dieser auf den ersten Blick sehr merkwürdige Fall dürfte sich vielleicht am einfachsten durch die Annahme erklären lassen, dass die Zellhaut unterhalb des Ringes von lebendem Plasma durchsetzt ist.

Dass Diatomeen auch in die Membran Eisen einlagern können, hat bereits Richter[1] betont und aus seiner Arbeit entnehme ich, dass auch Sjöstedt[2] bei den epiphytischen Diatomeen Cocconeis scutellum Ehg. var. ornata Grun. und C. pediculus Ehg. var. baltica (Dannf.) A. Cleve v. Euler Eisenauflagerung beobachtet hat.

f) Cocconeis als Manganorganismus.

Marine Algen sind oft mit einer ungeheuren Menge einer Cocconeis-Art bedeckt, die die Eigentümlichkeit besitzt, sich mit einer braunen Schichte von Manganoxyd zu umgeben. Peklo[3] hat eine solche Cocconeis auf einer marinen Cladophora gesehen.

Das Vorkommen solcher Cocconeis ist aber viel verbreiteter als man bisher angenommen hat; ich habe sie auf vielen Rot-und Braunalgen des Pacifik gefunden und nach dem, was ich in Sendai, Asamushi and Misaki gesehen, kann es keinem Zweifel unterliegen, dass diese Diatomee bei der Entmanganisierung des Meerwassers eine sehr bedeutende Rolle spielt. Auffallend ist, dass die Fällung des Mangans, abgesehen von kleinen Mengen weder ober-noch unterseits sondern fast ausschliesslich seitlich rings um die festsitzende Zelle erfolgt. Fig. 1 u. 2, Tafel IV. f, g, m.

Die Dicke der Manganschichte wechselt zwischen ½–8.5 μ.

Wenn viele Individuen dicht neben einander liegen, so findet sich, da alle oder fast alle von einer Manganschichte umgeben sind, ein braunes Mangannetz zwischen den Zellen, das sie mit einander verkittet.

Cocconeis ist eine epiphytisch lebende Diatomee und es ist mir sehr wahrscheinlich, dass die Umrahmung des Kieselpanzers mit dem braunen Erz wesentlich zur Befestigung der Alge auf dem Substrate beiträgt.

g) Anabaena sp.

Neben der Bententherme bei Yumoto fand ich auf einem Felsen, der von heissem Wasser (45°C) überrieselt wurde, eine Nostoccacee mit Grenzzellen. Sie bildet Räschen mit kurzen Fäden, die sich mit einer mehr oder minder dicken Eisenhülle umgeben. Diese wird besonders an der Spitze der Fäden oft so dick, dass der Algenfaden unsichtbar wird und, wie vererzt aussieht. Die Fäden werden dann sehr spröde und zerbrechen leicht in kleinere Stücke. Tafel III. Fig. 9.

---

[1] Richter O., Beiträge zur Eisenaufnahme durch technisch wichtige Fasern etc. Faserforschung II. Bd. 3. Heft. p. 204 Verlag Hirzel in Leiptzig.

[2] Sjöstedt G., Om Järnutfällning hos Hafsalger vid Skånes Kuster Särtryck ur „Botaniska Notiser" 1921, p. 101.

[3] Peklo F., Über eine manganspeichernde Diatomee. Österr botan. Zeitschr. Jg. 1909, Nro. 8.

Ein Überblick über die angeführten Eisenalgen zeigt, dass unter diesen Ödogonium als Eisenfäller eine nicht unbedeutende und Cocconeis als Manganfäller eine hervorragende Rolle spielt.

2. Flagellaten.

a) Anthophysa vegetans (O. F. M.) Stein.

Ich kenne diesen Organismus seit vielen Jahren, besonders von der alten Nutzwasserleitung in Prag her und weiss, welch grosse Rolle er bei der Fixierung des Eisens im Wasser spielt. Er ist in manchen Wässern in diesem Punkte sogar der typischen Ockerbakterie Chlamydothrix ochracea gleichzustellen. Anthophysa ist im Wasser der japanischen Reisfelder ungemein verbreitet und kann im Laboratorium leicht erhalten werden, wenn man das Wasser von Reisfeldern mit etwas Detritus der darin vorhandenen Wasserpflanzen in einem Glase stehen lässt und nach einigen Tagen untersucht. Er treten dann oft rostbraune Flocken auf, die sich bei mikroskopischer Beobachtung als die eisenreichen Skelette der Anthophysa entpuppen. Wenn die an den Spitzen der Skelette befindlichen Zellkolonien frei geworden sind und dann nur die Skelette übrigbleiben, so können diese leicht mit fädigen Eisenbakterien verwechselt werden.

Die Skelette treten nicht selten in so grosser Menge auf, dass Flocken von ½ cm Durchmesser entstehen, die nach und nach zu Boden sinken und hier einen tiefbraunen Belag bilden.

b) Spongomonas intestinum (Cienk.) S. Kent.

Auf den Wasserblättern der Salvinia natans in Gesellschaft mit dem Rotator Melicerta ringens und in Laboratoriumskulturen von Wasserpflanzen nicht selten; grosse, lange wurmförmige Kolonien bildend, deren feinkörnige Gallerte in Folge der Eisenspeicherung eine bräunliche Farbe annehmen. Unter ähnlichen Verhältnissen trat auch Spongomonas uvella Stein auf.

c) Lagynion Pascher.

Diese Gattung war mir schon in Europa, in Prag und Wien oft begegnet und hier in und um Sendai ist Lagynion ein ungemein weit verbreiteter Flagellat, der in seinem runden Gehäuse so reichlich Eisen einlagert, dass dieses schliesslich tief rostbraun wird. Er spielt bei der Fixierung des Eisens in süssem Wasser zweifellos eine sehr wichtige Rolle. Die Art, die ich in Japan regelmässig gefunden, ähnelt am meisten der von Naumann als Lagynion ovale benannten Form. Sie ist in der Abbildung II zu sehen. Lagynion sitzt auf vielen Wasserpflanzen auf aber ebenso häufig fand ich diese Gattung auf der Wasserhaut in den Kulturgefässen des Laboratorium; Formen mit lang ausgezogenem Gehäuse habe ich in Japan nur selten gesehen.

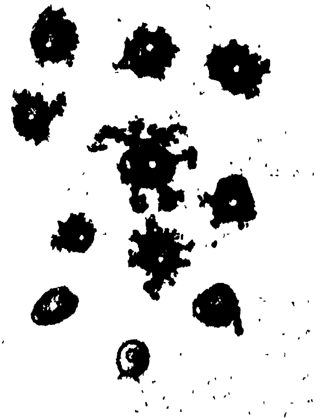


Abbildung II. Lagynion, mit Eisen inkrustierte Gehäuse. Vergr. 840.

d) Trachelomonas Ehrenberg.

Verschiedene Arten dieser Gattung sind gleichfalls eine häufige Erscheinung besonders im Frühjahr. Stehende Gewässer der Tümpel und Reisfelder enthalten sie oft, mitunter in sehr bedeutender Menge.

Unter den von mir beobachteten Flagellaten spielen jedenfalls Anthophysa, Trachelomonas und Lagynion als Eisenspeicherer eine bedeutende Rolle. Bei Trachelomonas konnte ich eine Beobachtung machen, die auf den ersten Blick rätselhaft erschien, die ich aber vollends aufklären konnte. Wenn man frische Trachelomonas auf dem Objektträger mit einem Tropfen einer 2% gelben Blutaugensalzlösung und einem Tröpfchen verd. Salzsäure behandelt, so färbt sich die mit Eisenoxyd incrustierte Wand tief blau und nach einiger Zeit oder gleichzeitig entsteht von der Wand aus eine Blase, ein Sack oder ein kurzer Schlauch von tief blauer Farbe. Tafel III, Fig. 8.

Dieser Sack oder Schlauch ist nichts anderes als eine Traubesche Zelle: Durch die Salzsäure wird das Eisen in Lösung gebracht und bildet sofort mit dem Blutlaugensalz eine semipermeable Niederschlagshaut von dem Ferrisalz der Ferrocyanwasserstoffsäure (Berlinerblau), die durch Intussusception ebenso zu einer Zelle heranwächst wie eine Membran von Ferrocyankupfer. Man kann sich auch leicht überzeugen, dass man ebenso wie mit Kupfersalzen auch mit Eisensalzen makroskopische, künstliche Zellen erzeugen kann, wenn man in eine 2% Ferrocyankaliumlösung kleine Kristalle von Eisensulfat fallen lässt. Nach mehrereren Stunden entstehen 1–4 cm lange, schlanke, schlauchförmige Zellen von Berlinerblau.

e) Rhipidodendron splendidum Stein.

Das wunderbare, fächerförmig verzweigte Skelet dieses Flagellaten hat eine braune Farbe, die durch oft hochgradige Eiseneinlagerung bedingt ist. Dieser Eisenorganismus findet sich oft in den Reisfeldern.

f)

Im Anschluss an die Flagellaten seien hier einige Beobachtungen über Eisenspeicherung durch einige niedere Tiere eingeschoben.

So fand ich auf den als Wurzel fungierenden Wasserblättern von Salvinia natans, die oft zu Millionen die Teiche und Reisfelder bedeckt, das epiphytisch lebende Rädertier Melicerta ringens (L.) Es bildet ein regelmässiges röhrenförmiges gegen die Basis sich etwas verchmälerndes Gehäuse. Dieses besteht aus dicht gelagerten Kügelchen, die dem Gehäuse eine zellenähnliche Struktur verleihen. Die Farbe des Gehäuses ist bräunlich oder braun. Ich vermutete, dass die braune Farbe auf einer eingelagerten Eisenverbindung beruht und die ausgeführten Reaktionen bestätigten diese Vermutung.

Ausserdem zeigten die Stiele mancher Ciliaten wie Vorticella und Carchesium oft starke Eiseneinlagerung.

3. HÖHERE PFLANZEN.

Die Eisenspeicherung in und auf der Membran, wie wir sie bisher bei Algen, Pilzen und Flagellaten kennen gelernt haben, ist aber nicht auf diese beschränkt, sondern findet sich auch bei manchen anderen ja auch bei höheren Wasserpflanzen. Ich habe diesen Gegenstand nicht in den Rahmen meiner japanischen Untersuchungen einbezogen und verweise daher auf meine einschlägigen Beobachtungen, die ich in Europa seinerzeit gemacht habe.[1] Nur auf einige besonders hochgradige Eisenspeicherungen sei hier vorübergehend aufmerksam gemacht.

a) Fontinalis antipyretica L.

Vor 34 Jahren habe ich mich bereits von dem grossen Speicherungsvermögen dieses Laubmooses überzeugt. Das Eisen liegt hier in den Membranen und zwar in so grossen Mengen, dass in der Asche Eisenskelette zurückleiben. Auch in Japan erwies sich dieses Moos als bedeutender Eisenspeicher.

b) Trapa natans L.

Dass die Fruchtschale der Wassernuss ein ungemein eisenreiches Gewebe darstellt, habe ich bereits vor langer Zeit[2] beschrieben. Dasselbe zeigte mir auch die Wassernuss in Japan. Das Eisen kommt hier in locker gebundener Form vor und kann teilweise durch heisses Wasser leicht ausgezogen werden. Der Eisenreichtum ist so gross, dass die Asche des Perikarps braun wie Eisenrost aussieht. Vgl. auch. p. 157.

c) Hydrilla verticillata Royl. var. Roxburghii Casp.

Ich[3] habe seinerzeit gezeigt, dass gewisse Wasserpflanzen, wenn sie in verdünnten Lösungen verschiedener Mangansalze z. B. in einer 0.1 prozentigen Lösung von Manganochlorid im starken Lichte gezogen werden, eine braune Manganverbindung in der äusseren Wand der Epidermisszellen in sehr charakteristischer Weise niederschlagen und sich dabei braun färben. Besonders schön gelingen solche Versuche mit Elodea canadensis, einer Pflanze, die auffallender Weise bisher in Japan noch nirgends gefunden wurde, doch zeigen auch andere Wasserpflanzen wie Vallisneria spiralis, Ranunculus aquatilis und Myriophyllum verticillatum dasselbe. Perušek[1] hat später unter meiner Leitung noch andere Wasserpflanzen ausfindig gemacht, die Manganeinlagerung in die Membran zeigen.

---

[1] Molisch H., Die Pflanze in ihren Beziehungen zum Eisen. Jena 1892, p. 30.

[2] Molisch H., l. c. p. 39.

[3] Molisch H., Über lokale Menbranfärbung durch Manganverbindungen bei einigen Wasserpflanzen. Sitzber der Kais. Akad. d. Wissensch. in Wien Bd. CXVIII. Abt. I. Nov. 1909. p. 1427.

Blickt man von oben auf die Oberhaut der Blätter herab, so glaubt man, dass der Zellinhalt braun und wie in Plasmolyse zurückgezogen erscheint. Doch dies ist eine Täuschung. Die braune mehr oder minder elliptische Figur, die man sieht, gehört gar nicht dem Zellinhalt an, sondern ausschliesslich der Wand und zwar der äussern Wand der oberen Epidermiszellen, Die entprechenden Zellen der Unterseite zeigen diese Erscheinung nicht oder in viel geringerem Grade.

Dieses Niederschlagen von Manganoxyd in der Zellhaut verdient die Aufmerksamkeit der Physiologen aus zweierlei Gründen. Erstens lehrt das Phänomen, dass an der Stelle der Einlagerung in der Zellhaut andere Verhältnisse herrschen müssen als knapp daneben oder an den anderen Wänden der Oberhautzelle. Zweitens vollzieht sich die Einlagerung nur im Lichte. Wir müssen daher annehmen, dass unter dem Einflusse des Lichtes an der Ablagerungsstelle in der Membran ganz bestimmte Vorgänge platzgreifen müssen, die zur Manganeinlagerung führen.

Die geschilderten Verhältnisse beziehen sich ausschliesslich auf Kulturversuche, es liegt aber kein Grund vor, dass dasselbe nicht auch in der Natur vorkommt, wenn Mangansalze der natürlichen Wässer in genügender Menge gelöst sind. Dies konnte ich tatsächlich in Japan beobachten, Am 9. September 1923 sammelte ich in einem Wassergraben beim Exerzierplatz in Sendai nahe der über den Hirose-Fluss führenden Brücke Hydrilla, deren Blätter an älteren Sprossen mir durch ihre bräunliche oder braune Farbe auffielen. Als ich diese mikroskopisch untersuchte, fand ich fast alle oberen Epidermiszellwände mit tiefbraunen Ellipsen versehen, Fig. 3, Taf. IV., so wie ich es bei den vorhin besprochenen Mangankulturversuchen beobachtet habe. Die mikrochemische Prüfung ergab, dass die braunen elliptischen Membranflecke hauptsächlich aus einer braunen Manganverbindung mit etwas Eisen bestanden.

---

[1] Perušek E., Über Manganspeicherung in den Membranen von Wasserpflanzen. Sitzb. d. Akad. d. Wiss. in Wien., mathem. naturw. Kl. Abt. I. 1919 Bd. 128.

Die Blätter der Hydrilla waren überdies mit zahlreichen Oedogoniumkeimlingen besiedelt, die ihrerseits vielfach mit Eisenoxyd beschlagen waren. Ausserdem sassen auf den Blättern Rotatorien der Gattung Melicerta fest, deren röhrenförmiges Gehäuse von eingelagertem Eisen braun war.

d) Oryza sativa L.

Zu wiederholten Malen war mir aufgefallen, dass, wenn ich junge Reispflanzen auf dem Reisfelde aus dem nassen Boden zog, die Wurzeln abgesehen von den jüngsten Teilen rostbraunn gefärbt waren. Die Ursache der Braunfärbung war Eisenoxyd, das in Form einer bräunlichen Masse auf der Oberfläche der Wurzel niedergeschlagen ist. Die Wurzelhaare erschienen deutlich mit Eisenoxydhydrat bedeckt, überdies enthielt auch ihre Membran viel locker gebundenes Eisen. In noch viel auffallenderer Weise sind die Wurzeln schon fruchttragender Pflanzen mit Eisenosyd bedeckt und daher tief rostbraun.

Obwol das Wasser der Reisfelder in Japan oft reich an Eisen ist, so muss es doch als eine auffallende Eigenschaft der Reiswurzel hingestellt werden, Eisen an ihrer Oberfläche niederzuschlagen, da andere im selben Boden gleichzeitig wachsende Pflanzen keine Braunfärbung ihrer Wurzeloberfläche erkennen lassen. Es wäre sehr wünschenswert, diese Erscheinung weiter zu untersuchen.

4. Über postmortale Eisenspeicherung.

Wenn man in Spätherbste die abgeernteten Reisfelder betrachtet, so findet man häufig Potamogeton natans mit abgestorbenen kohlschwarzen Blättern.

Die Blätter sind entweder ganz schwarz oder schwarz und braun. Die kohlschwarze Farbe fiel mir auf und eine nähere mikrochemische Untersuchung ergab, dass solche Blätter von einer Eisenverbindung geradezu strotzen. Unterwirft man ein Stück eines solchen Blattes der Eisenreaktion mit Ferrocyankalium unter dem Mikroskop, so färbt sich nicht bloss der Schnitt sondern die ganze nähere Umgebung bis zur Undurchsichtigkeit tiefblau, wobei ein reichlicher Niederschlag von Berlinerblau entsteht. Verascht man ein solches schwarzes Blatt, so erhält man eine tief rostbraune Asche, die, mikroskopisch geprüft, das Gewebe des Blattes als ein Eisenskelett erscheinen lässt.

Mikroskopische Schnitte lassen erkennen, dass insbesondere der Inhalt der Epidermiszellen der Oberseite des Blattes und der die Lakunen des Mesophylls begrenzenden Zellen blauschwarz oder schmutzig schwarzbraun gefärbt ist und dass darauf die schwarze Farbe des toten Blattes beruht. Die Eisenreaction rührt von diesem bläulich schwarzen Körper her und dieser ist wohl nichts anderes als gerbsaures Eisen.

Die Blätter von Potamogeton sind im lebende Zustande sehr gerbstoffreich und wenn sie absterben, was im Herbste normal der Fall ist, so wird das in die toten Zellen eintretende Eisen als gerbsaures Eisen gefällt und dies geschieht so lange, bis alle Gerbsäure abgesättigt ist. Auf diese Weise kommt es zu einer so erstaunlichen Eisenanhäufung, dass die Blätter ganz schwarz werden und beim Verbrennen Eisenskelette hinterlassen.

Wenn man Wassernüsse (Trapa) noch vor ihrer völligen Reife betrachtet, so sind sie gewöhnlich nicht schwarz sondern hellbraun, erst später, wenn sie längere Zeit in eisenhältigem Wasser liegen, werden sie schwarz schliesslich kohlschwarz. Der Vorgang ist meiner Meinung in wesentlichen derselbe wie bei Potamogeton, nur ist hinzuzufügen, dass die Fruchtschale, wenn sie noch braun ist, auch schon viel Eisen enthält.

Mir fiel auch schon in Europa auf, dass die Samenschalen von Aesculus Hippocastanum, die bekanntlich eine braune Farbe haben, nach und nach, wenn sie lange Zeit im Wasser liegen, in Folge der Bildung von gerbsaurem Eisen eine tief schwarze Farbe annehmen.

Man kann diese Schwarzfärbung in kurzer Zeit und zwar schon nach wenigen Tagen hervorrufen, wofern man reife braune Rosskastanien-Samen in einer sehr verdünnten Lösung von Eisenchlorid oder Eisensulfat liegen lässt. Am Meeresstrande des Pacifik bei Arahama nahe bei Sendai fand ich im Sande viele Samenschalen von Castanea vesca, die kohlschwarz waren. Sie lagen seit vorigem Jahre im Sande, hatten postmortal Eisen aufgenommen und durch Bildung von Gerbstoffeisen die schwarze Farbe angenommen. Die schwarze Schale ist reich an Eisen und dies gibt sich ebenso wie bei Trapa auch durch die rostbraune Farbe der Asche zu erkennen.

Es ist selbstverständlich, dass die schwarze Farbe eines abgestorbenen Organs auch in anderer Weise zustandekommen kann z. B.

durch Oxydation eines Chromogens, doch dies hat mit einer Eisenspeicherung nichts zu tun.

Betrachtet man der Atmosphäre und dem Regen ausgesetzte Bretterwände oder Holzzäune, in denen eiserne Nägel eingeschlagen sind, so bemerkt man oft einen vom Nagel nach abwärts ziehenden, mehrere Centimeter langen schwärzlichen Streifen. Auch dieser ist auf eine lokale Eisenspeicherung im Holze zurückzuführen. Das Eisen des Nagels wird durch die Kohlensäure des Regenwassers in geringen Mengen in Lösung gebracht; diese Eisenspuren reagieren mit dem Gerbstoff der Holzzellen und veranlassen die Bildung von dunklem Gerbstoffeisen in Form des schwärzlichen Streifens.

Diese postmortale Bindung des Eisens durch Gerbstoffe der Pflanze spielt in der Natur eine nicht unbedeutende Rolle und es ist mir wahrscheinlich, dass der auffallend grosse Eisengehalt mancher Kohlearten und mancher pflanzlicher und tierischer Fossilien auf postmortale Fällungen des Eisens zurückzuführen ist.

5. RÜCKBLICK.

Wenn wir die erhaltenen Ergebnisse überschauen, so können wir sagen, dass die meisten Eisenorganismen, die sich in Europa vorfinden, von mir auch in Japan und zwar in weiter Verbreitung festgestellt worden sind. Besonders diejenigen, die bei der Speicherung und Fällung des Eisens eine so grosse Rolle spielen, wie die fädigen Eisenbakterien, allen voran Chlamydothrix ochracea, Chl. sideropous, Gallionella ferruginea und unter den nicht fädigen Siderocapsa erfreuen sich einer grossen Verbreitung. Dasselbe gilt auch von eisenliebenden und eisenspeichernden Algen und Flagellaten, unter denen besonders Closterien, Oedogonien, Characium, Anthophysa und Lagynion zu nennen sind. Hiezu kommt der Einfluss höherer Wasserpflanzen auf die Fällung des Eisens uud nicht zuletzt auch die postmortale Speicherung des Eisens durch gewisse Pflanzen insbesondere gerbstoffhältiger.

Vom neuen hat sich bei der Feststellung mikroskopischer Eisenorganismen gezeigt, dass den Mikroorganismen eine weite Verbreitung auf unserm Erdball zukommt und dass sie zum Kosmopolitismus neigen, wie ich noch an einem anderen Orte ausführlicher erörtern werde.

III.

ÜBER EISENBACTERIEN, DIE AUS ORGANISCHEN EISENVERBINDUNGEN EISEN FÄLLEN.

In meinen beiden Schriften über die Pflanze in ihren Beziehungen zum Eisen und über Eisenbakterien habe ich zu wiederholten Malen betont, dass die Eisenbakterien und auch andere Eisenorganismen beim Niederschlagen des Eisens in der Natur eine wichtige Rolle spielen, dass sie sich auch an der Bildung der Rasenerze beteiligen können und in einer speziellen Schrift[1] habe ich gezeigt, dass auch phanerogame Wasserpflanzen die Fällung von gelöstem Eisen veranlassen können.

a) Harders Versuche

In einer vor wenigen Jahren erschienenen Arbeit hat Harder[2] die chemischen und geologischen Einflüsse auf die Fällung des Eisens in Beziehung zur Geologie auf Grund der vorhandenen Literatur und eigener Erfahrungen übersichtlich zusammengestellt und auch die wichtige Beobachtung gemacht, dass in der Natur Kokken und stäbchenartige Bakterien weit verbreitet sind, die in einer Lösung von zitronsaurem Eisen-Ammon (ferric ammonium citrate) nach einiger Zeit Eisenoxyhydrat fällen. Er ging in folgender Weise vor: Zitronsaures Eisenammon wurde in der Menge von ½ Prozent in destilliertem Wasser, in gewöhnlichem Wasser, in Wasser von einem See (Mendota), von einer Eisenquelle und in einem Wasser mit etwas Gartenboden gelöst. Mit diesen Lösungen wurden je 2 Kolben mit je 500 cm^3 gefällt, wovon der eine sterilisiert der andere zur Kontrolle nicht sterilisiert wurde.

Wenn diese Kolben bei Zimmertemperatur längere Zeit standen, so zeigte sich folgendes. Die Lösungen der sterilisierten Kolben zeigten keine Fällung, hingegen war in den nicht sterilisierten mit Ausnahme der Lösung in destilliertem Wasser das Eisen überall vollständig aus gefällt worden. Die Fällung erfolgte in den Gefässen nach verschiedener Zeit. In Leitungswasser nach 2 Monaten, im Wasser vom See Mendota nach etwa 7 Wochen, in Wasser der Eisenquelle nach etwa 1 Monat und in dem mit etwas Erde versetzten Kolben nach beiläufig 2 Wochen. Damit war der Beweis geliefert, dass bei Anwendung von zitronsaurem Eisen-Ammon die Fällung des Eisens höchstwahrscheinlich biologischen Vorgängen zuzuschreiben ist.

---

[1] Molisch H., Über die Fällung des Eisens durch das Licht und grüne Wasserpflanzen Sitzber. d. k. Akad. d. Wissensch in Wien. Bd. CXIX. Abt. 1. 1910, p. 959.

[2] Harder E. C., Iron-Depositing Bacteria and their Geologic Relations. Washington 1919. Department of the Interior. United States Geological Survey. Professional paper 113.

Der Einwand, dass die Fällung in den sterilisierten Wässern vielleicht deshalb ausgeblieben ist, weil durch das Erhitzen das Eisensalz irgend eine Veränderung erlitten haben konnte, die die Hydrolyse verhinderte, wurde durch folgenden Versuch entkräftet. Nachdem die sterilen Lösungen schon 4 Monate gestanden waren, wurden sie mit etwas frischem Bodeninfuss geimpft und schon nach sehr kurzer Zeit zeigte sich die Praecipitation. Damit war also bewiesen, dass die Fällung auf biologische Einflüsse zurückzuführen ist.

Scheinbar in Widerspruch damit steht die Beobachtung Harders, dass gegen alle Erwartung in dem Eisenoxydhydratniederschlag keine Spur von Eisenbakterien zu sehen war, abgesehen von dem Wasser der Eisenquelle, wo Leptothrix ochracea sich vorfand. Hier waren sie aber nach der Meinung Harders schon in dem verwendeten Wasser am Beginne des Versuchs zugegen. Also abgesehen von dieser Ausnahme waren Bakterien in der kolloidalen, texturlosen Masse nicht erkennbar. („in which bacterial forms are unrecognizable"[1]

Obwohl also Bakterien nicht erkennbar waren, nimmt Harder doch an, dass sie vorhanden sind und das Eisen fällen. Um nun diese Bakterien aufzufinden, versuchte er sie zu isolieren und hatte schliesslich Erfolg, als er ein Nährsubstrat verwendete, bestehend aus Heyden-Nährstoff-Agar und 1% Eisenammoncitrat. Dieses Substrat wurde mit einer Bodeninfusion geimpft, in Platten ausgegossen und dann im Brutofen bei 28°C belassen. Es entwickelten sich viele verschiedene Kolonien, darunter tauchten schon nach dem 2. Tage auch Kolonien mit einem braunen Hof auf, die nach 5–8 Tagen in folge von Fällung des Eisenoxydhydrats dunkelbraun mit irisierender Oberfläche wurden.

Unter den erhaltenen braunen Kolonien war die typische, eine unregelmässige, flache Kolonie mit zerklüfteten, runzeligen oder federartigem Rand, dem Agar meist aufliegend. Diese Kolonien fällten Eisenoxydhydrat fast unmittelbar nach Beginn ihrer Entwicklung und bedeckten sich nach 5-6 Tagen mit einer spröden, dunkelbraunen Kruste von Eisenoxydhydrat.

---

[1] Harder E. C., l. c. p. 33.

Neben diesen Kolonien tauchten noch verschieden andere auf, die gleichfalls Eisen niederschlugen. Sie bestanden aus Bakterien von Stäbchen- und Kokkenform und manche aus Actinomyces.

Harder hat auch, abgesehen von Eisenammoncitrat, noch andere organische Eisenverbindungen in derselben Weise geprüft und nach Impfung mit Bodeninfusionen gleichfalls Fällungen erhalten. So mit Eisencitrat, Eisenoxalat, Eisenlaktat und Eisenacetat. In Mangansalzen unterblieben die Fällungen, doch entwickelten sich Schimmelpilze z. B. in Mangancitrat.

Die Versuche Harders, in anorganischen Eisenlösungen Fällungen des Eisens durch Bakterien hervorzurufen, schlugen insgesamt fehl, weil schon durch rein chemische Prozesse, durch Oxydation und Hydrolyse die Fällung des Eisens erfolgte.

Von Wichtigkeit erschien mir der Nachweis Harders, dass sterilisierte organische Eisenlösungen insbesondere Eisenammoncitrat nach einiger Zeit keinen Niederschlag von Eisenoxydhydrat erzeugen, während nicht sterilisierte dies tun. Auffallend ist nur, dass Harder in dem Eisenniederschlag und überhaupt in den unsterilisierten Lösungen direkt keine Bakterien beobachten konnte. Es müsste also angenommen werden, dass zwar Bakterien da waren, aber so spärlich, dass sie nicht aufgefunden werden konnten. Doch das ist mit Rücksicht auf die grosse Menge des Praezipitats nicht gerade wahrscheinlich. Oder man muss annehmen, wenn hier biologische Vorgänge überhaupt eine Rolle spielen, dass es sich um unsichtbare, um sogenannte ultramikroskopische Bakterien handelt. Auch das erscheint nicht wahrscheinlich.

Um über diesen dunklen Punkt ins Klare zu kommen und mir ein eigenes Urteil bilden zu können, wiederholte und erweiterte ich den Versuch Harders.

b) Eigene Versuche

über die Fällung des Eisens in Eisen-ammon-citrat Lösungen durch Bakterien.

Je zwei Probegläser wurden zu je 50 cm^3 gefüllt:

mit destilliertem Wasser (I)
Leitungswasser der Stadt Sendai (II)
Brunnenwasser der Universität Sendai (III)
Wasser einer Eisenquelle i. d. Nähe von Sendai (IV)
Meerwasser von Matsushima (V)

Jedem Probeglas wurden 0.25 g Eisenammoncitrat beigefügt. Je ein Kolben der verschiedenen Wässer (I–V) wurde sterilisiert, der andere nicht, er diente zur Kontroll. Die Sterilisierung wurde zweimal durchgeführt, das erstemal 1 Stunde und das zweitemal nach 1 Tage ½ Stunde. Die Lösungen waren nach edr Sterilisierung vollkommen klar, sie waren nur etwas dunkler als die unsterilisierten. Sodann wurden alle Kolben bei Zimmertemperatur in Finstern aufgestellt.

Der Versuch begann am 18. XI. 23.
Am 23. XI. also nach 5 Tagen waren
alle sterilisierten Wässer (I–V) unverändert und klar,
Alle unsterilisierten trüb.
Bei I war die Trübung schwach, oben etwas stärker als unten.
Bei II Trübung deutlich.
Bei III. „ „
Bei IV war die Trübung oben stärker.
Bei V gleichmässige Trübung.
Am 5. Dezember also nach 17 Tagen waren
alle sterilisierten (I-V) unverändert und klar,
alle unterilisierten trüb.

Bei II, IV und V hatte die Trübung zugenommen, oben hat sich eine braune Haut gebildet und bei III war bereits fast alles Eisen als brauner Niederschlag ausgefallen, oben ein ziemlich dickes Häutchen und unten eine ½ cm hohe Schichte bildend. In Folge dessen war Lösung bei III schon fast farblos geworden.

Um zu sehen, ob sich in den nicht sterilisierten Proberöhren Bakterien oder andere Organismen entwickelt hatten, wurden kleine Mengen der Flüssigkeit und des Niederschlags steril den Röhren entnommen und mikroskopisch geprüft. Dabei ergab sich folgendes.

Röhre I. Die geschlossene Haut oben bestand fast aus einer Reinkultur einer Unzahl ziemlich grosser Stäbchenbakterien, von denen ein Bruchteil in lebhaft aktiver Bewegung war. Zwischen den Bakterien lagen, ziemlich gleichmässig verteilt, braune Bröckchen von Eisenoxydhydrat. Diese gaben prompt die Eisenreaction, die Bakterien selbst nicht oder nur schwach. In der Lösung mehr unten waren dieselben Bakterien, aber spärlich.

Röhre II. Die dicke braune Haut oben besteht aus einer Stäbchen-Kapselbakterie. Die Kapsel ist kreisrund oder elliptisch und 6–9 μ im Durchmesser. Die Bakterie selbst 2–3.5 μ lang und 0.5–0.8 μ dick. Die Kapsel gibt intensive Eisenreaction desgleichen die Bakterie selbst. Behandelt man mit Methylenblau, so färbt sich der Rand der Kapsel und die Bakterie stärker als die dazwischen liegende Kapselmasse.

Röhre III. Oben befindet sich eine dicke braune Haut, unten ein bis 1 cm hoher Niederschlag von rostbrauner Farbe. Die Haut wird von zwei Bakterien gebildet, einer plumpen, mitunter spindelförmigen und einer sehr schmalen Stäbchenbakterie. Ausserdem fanden sich Amoeben und reichlich eine Art von Infusorien vor. Die dicke Bakterie liegt in Schleim eingebettet vor. Auf und zwischen diesen Organismen befindet sich eine dicke Schichte von Eisenoxydhydrat. Diese und der Schleim geben Eisenreaction, die Bakterie selbst nicht oder wenig.

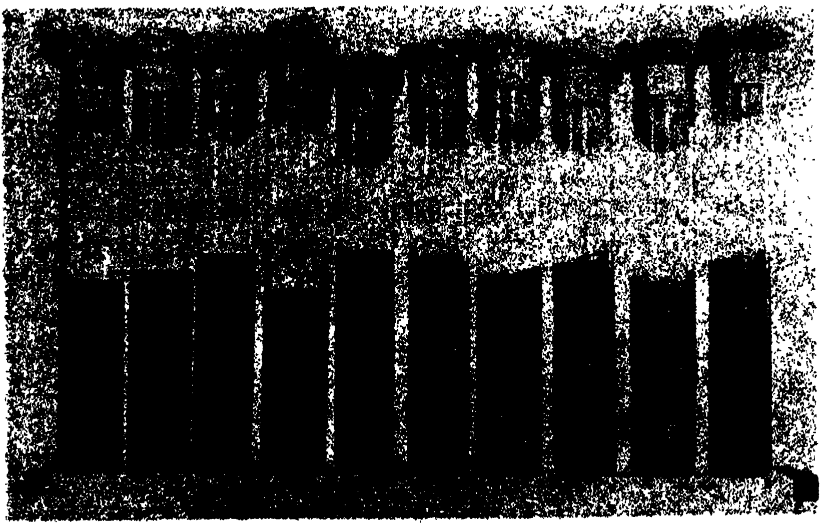


Abbildung III.
Versuch über die Fällung des Eisens aus Lösungen von Eisenammoncitrat durch Bakterien.
Links: Die Proberöhren 1–5, sterilisiert, zeigen keine Fällung.
Rechts: Die Proberöhren 1–5, nicht sterilisiert, zeigen überall Fällung.

Röhre IV. Oben eine braune Haut, unten eine dünne Schicht braunen Niederschlags. Die Haut besteht aus einer plumpen, kurzen stäbchen-bis spindelförmigen Bakterie. Sie liegt im Schleim, der die Eisenreaction gibt.

Röhre V. Oben eine dünne Haut, bestehend aus einer ziemlich langen, unbeweglichen Stäbchenbakterie.

Als ich etwa 10 Monate später am 21. September 1924 den Versuch wieder kontrollierte, waren noch immer alle sterilisierten Lösungen völlig klar, in den unsterilisierten war alles Eisen ausgefallen. Die Versuche wurden mehrmals wiederholt, stets mit demselben Ergebnis. Dabei wurde auch die Tatsache, dass nicht bloss das süsse sondern auch das Meerwasser stets Bakterien enthält, die das Eisen des zitronsauren Eisen-Ammon fällen, über jeden Zweifel erhoben.

c) Ergebnisse.

Die geschilderten Versuche haben unter anderen unzweideutig 2 wichtige Tatsachen ergeben:

1. In sterilisierten Lösungen von Eisenammoncitrat in verschiedenen Wässern erfolgt keine Fällung des Eisens. Solche Lösungen bleiben vollständig unverändert klar und bakterienfrei.

2. Unsterilisierte Lösungen derselben Art trüben sich und lassen schliesslich das Eisen in Form eines reichlichen Niederschlags von Eisenoxydhydrat ausfallen.

Dieses Resultat stimmt mit den Experimenten von Harder. Hingegen weicht das Ergebnis meiner Versuche insoferne ab, als bei meinen Versuchen in den nicht sterilisierten Wässern stets reichlich Bakterien auftraten, die zweifellos die Fällung des Eisens veranlassen. Wie dieser Gegensatz zu erklären ist, kann ich nicht bestimmt sagen, doch scheint es mir nicht unwahrscheinlich, dass Harder erst an Ende des Versuchs nach Bakterien gesucht und diese in dem überaus reichlichen Niederschlag übersehen hat. Ich empfehle daher jenen, die solche Versuche wiederholen wollen, besonders die am Meniscus entstehende braune Haut zu untersuchen, denn diese bildet oft eine geschlossene Decke von Bakterien.

Die hier auftretenden Bakterien sind von verschiedener Art; manche nehmen in ihrer Kapsel oder in ihren Zellen Eisen ziemlich reichlich auf, manche nicht oder nur wenig. Die Hauptmasse des sich am Boden absetzenden Niederschlags besteht aus Eisenoxydhydrat und daher muss man annehmen, dass die Eisenfällung der Hauptsache nach eine extracelluläre ist und durch Bakterien erfolgt.

Da nun die Eisenfällung in den verschiedensten Wässern ja, wie ich gezeigt habe, sogar im Meerwasser vorsichgeht, so müssen derartige eisenfällende Bakterien in der Natur ungemein verbreitet sein und im Kreislauf des Eisens eine grosse Rolle spielen.

Die bisherigen Versuche lassen noch die Möglichkeit offen, dass auch Schimmelpilze die Fähigkeit haben könnten, Eisen aus citronsaurem Eisen-Ammon zu fällen. Es wurde daher noch ein neuer Versuch derselben Art wie früher gemacht, aber mit Reinkulturen von Penicillium und Aspergillus.

Beginn am 8. März 1924.

Nro 1. Leitungswasser + zitr. Eisen-Ammon, nicht geimpft.
Nro 2. Leitungswasser + zitr. Eisen-Ammon mit Penicillium geimpft
„ 3. „ + „ + Pepton, mit Penicillium geimpft
„ 4. Meerwasser + „ + „ , nicht „
„ 5. „ + „ + „ , mit Penicillium geimpft
„ 6. Leitungswasser + „ + „ , mit Aspergillus „
„ 7. „ + „ + „ , nicht „

Alle Gefässe wurden mit 5% zitronsaurem Eisenammon versetzt, sterilisiert und dann geimpft. Die Peptongläser enthielten 0.3% Pepton. Alle Gefässe wurden bei Zimmertemperatur im Finstern aufgestellt.

Am 6. April 1924 zeigte sich folgendes.

Nro 1. Lösung klar, keine Fällung, steril
„ 2. „ „ „ „ Penicillium üppig entwickelt
„ 3. „ „ „ „ „ „ „
„ 4. „ „ „ „ steril
„ 5. „ „ „ „ Penicillium üppig entwickelt
„ 6. „ „ „ „ Aspergillus „ „
„ 7. „ „ „ „ steril

Am 22. IX. 24. war alles so wie vorher.

Dieser Versuch und seine Wiederholungen lehrten, dass die von mir verwendeten Arten von Penicillium und Aspergillus nicht die Fähigkeit haben, Eisen aus der genannten organischen Verbindung zu fällen und dass sterile Lösungen gleichfalls klar bleiben.

Wenn die eben geschilderten Versuche beweisend sein sollen, müssen sie, wie ich dies getan habe, bei Abschluss von Licht gemacht werden, weil ich[1] seinerzeit gefunden habe, dass das Licht das Eisen gewisser verdünnter Eisenlösungen zu fällen vermag. Wird z. B. eine verdünnte (0.0068%) Lösung von zitronsaurem Eisenammon, zitronsaurem Eisenkalium oder von zitronsaurem Eisen belichtet und unbelichtet aufgestellt, so wird das Eisen innerhalb einer gewissen Versuchszeit nur im Lichte gefällt.

Ferner wurde von mir gezeigt, dass nicht bloss das Licht an und für sich sondern auch höhere Wasserpflanzen (Elodea, Stratiotes, Vallisneria etc.) die Fällung des Eisens günstig beeinflussen, wenn es z. B. in Form des Ferricarbonates, essigsauren und zitronsauren Eisens geboten wird. Es ist mir in hohem Grade wahrscheinlich, dass diese Tatsache mit der Fällung des kohlensauren Kalkes[2] und mit der Sauerstoffentwicklung der Wasserpflanzen bei der Kohlensäure-Assimilation in Lichte zusammenhängt. Der kohlensaure Kalk reagiert alkalisch und wird daher Eisen niederschlagen und der im Lichte durch die grüne Wasserpflanze entbundene Sauerstoff wird die Oxydation des gebildeten Eisenoxyduls zu Eisenoxyd beschleunigen.

Dass das Eisen ebenso wie das Mangan in der Oxydform in ganz eigenartiger Weise in der Aussenhaut der Epidermis der Blattoberseite eingebettet wird und dass dieser Vorgang sich nur im Lichte vollzieht, wurde bereits auf. p. 154-155 erwähnt.

d) Rückblick.

So sehen wir denn, dass in der Natur ein Heer von bestimmten Bakterien, Algen und anderen Kleinwesen Eisen meist in Form von Eisenoxydhydrat niederschlägt und zweifellos an der Bildung von Eisenoxydablagerungen einen grossen Anteil hat.

---

[1] Molisch H., Über die Fällung des Eisens durch das Licht und grüne Wasserpflanzen. Sitzber. d. kais. Akad. d. Wiss. in Wien. Bd. CXIX. Abt. 1. p. 959.

[2] Ruttner Fr., Das elektrolytische Leitvermögen verdünnter Lösungen unter dem Einfluss submerser Gewächse. Sitzber. d. Akad. d. Wiss. Wien, mathem-naturw. Kl. 1. 1921. 130 Bd. p. 71-108.

Unterstützt wird die Tätigkeit dieser Kleinwesen noch durch das Licht und durch verschiedene Wasserpflanzen.

Das geflügelte Wort „Kleine Ursachen, grosse Wirkungen" gilt nicht nur von dem Aufbau der Korallenriffe, der Erdkreide oder dem Kieselgur, dem Lithothamnion- und Foraminiferenkalk sondern auch von der Bildung der Sumpf- und Rasenerze und anderer Eisenoxydablagerungen, an denen der Boden Japans so reich ist. Die rostrote Farbe so vieler Böden in Japan, die sogar dem Laien auffällt, verdanken ihrer Ursprung nicht bloss rein physikalis-chchemischen sondern und zwar in hervorragendem Maaase auch biologischen Prozessen. Der grosse Anteil, der gewissen weit verbreiteten, ja man kann sagen, kosmopolitischen Lebewesen bei dem Zustandekommen der Eisendepots in der Natur zufällt, tritt mit fortschreitenden Untersuchungen immer mehr und mehr hervor und ist auch aus den Arbeiten Naumanns[1] und Harders[2] zu ersehen. Die in verschiedenen Ländern bisher gewonnenen Ergebnisse verdienen nicht nur die Aufmerksamkeit der Biologen sondern auch der Mineralogen und Geologen.

Die vorstehenden Studien erforderten weite Reisen in ganz Japan von Süden in Hondo bis Sachalin im Norden und ausserdem mehrere wertvolle Einrichtungen im Laboratorium. Die Durchführung solcher Reisen wäre nicht möglich gewesen, wenn die Saito-Gesellschaft Hoonkwai in Sendai nicht die Güte gehabt hätte, mir eine entsprechende Subvention für diese Arbeit zu verleihen. Daher fühle ich mich gedrängt, am Schlusse meiner Untersuchungen der Saito-Gesellschaft für die hochherzige Unterstützung meinen tiegefühlten Dank auszusprechen.

---

[1] Naumann E., l. c.

[2] Harder E. C., l. c.

ERKLÄRUNG DER TAFELN.

Tafel I.

Fig. 1. Anthophysa vegetans. Skeletstiele reichlich mit Eisenoxyd inkrustiert. Stark vergrössert.

Fig. 2. Anthophysa vegetans, Skeletstiel mit Kolonien. Stärker vergrössert. .Vergr. 500.

Fig. 3. Chlamydothrix ochracea. Leere Scheiden, dazwischen Brocken von Eisenoxydhydrat. Stark vergrössert.

Fig. 4. Clonothrix fusca. Bei v Verzweigung eines Fadens. Vergr. 312.

Fig. 5. Gallionella ferruginea. Fäden zylindrisch, typische Form, a alter stark vererzter Faden. Vergr. 840.

Fig. 6. Dasselbe, Fäden flach (Spirophyllum ferrugineum Ellis), a alter stark vererzter Faden. Vergr. 840.

Tafel II.

Fig. 1-5. Toxothrix ferruginea n. g. Verschieden alte Stadien dieser Eisenbakterie. 1 jung, 4-5 älter, 5 stark vererzt. Vergr. bei 1-4, 840, bei 5, 500.

Fig. 6. Siderocapsa Treubii. a, b, c, einzelne vererzte Haftscheiben, d vereinigte Haftscheiben. In der Mitte der hellen Höfe die Bakterien. Verg. 840.

Fig. 7. Chlamydothrix sideropous. a junge, b ältere, c alte Fäden mit vereinigten Haftscheiben. d Haftscheibe in Profil, e Ansatztelle des abgefallenen Fadens. Verg. 840.

Tafel III.

Fig. 1. Pleurotaenium nodulosum. a mit rostrotem Eisengürtel g. b daselbe. Vergr. 103.

Fig. 2. Desmidiacee mit ähnlichem Eisenring wie in Fig. 1. Vergr. 125.

Fig. 3. Surirella sp. Bedeckt mit einer Lage von Eisenoxyd. Vergr. 500.

Fig. 4. Characium mit brauner Eisenhaftscheibe. Vergr. 500.

Fig. 5. Oedogonium-Keimlinge bei a, b und c, den Beginn der Haftscheibenbildung und gleichzeitig Vererzung zeigend. Vergr. 82.

Fig. 6. Closterium-Arten nach Ausführung der Eisenreaction mit g. Blutlaugensalz. Bei a zeigt die alte Hälfte der Wand sehr schön den reichen Eisengehalt in Gegensatz zur jungen Hälfte. Vergr. 500.

Fig. 7. Zwei Trachelomonas-Arten a und b. Vergr. 500.

Fig. 8. Trachelomonas sp. bildet nach der Eisenreaction Traubesche Zellen (Niederschlagsmembranen von Berlinerblau) Vergr. 500.

Fig. 9. Nostoccacee, oben ohne, unten mit Eisenincrustation. Vergr. 500.

Tafel IV.

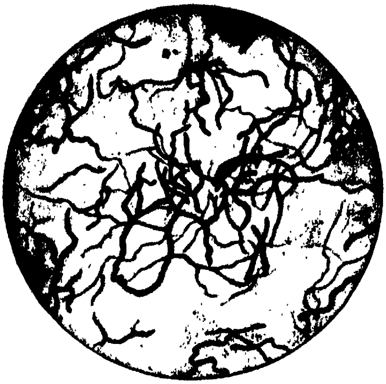
Fig. 1. Auf einem Thallusstück einer Floridee sitzen viele Cocconeis g auf, seitlich umgeben von einem Panzer aus braunem Manganoxyd. Vergr. 500.

Fig. 2. Zwei Cocconeis mit Manganpanzer. Vergr. 840.

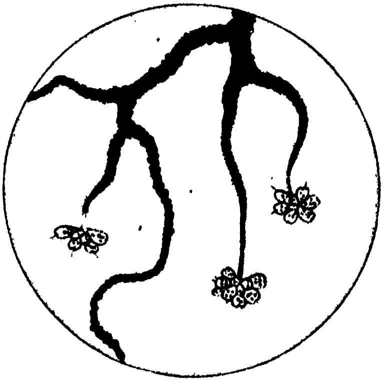
Fig. 3. Hydrilla verticillata Royl. var. Roxburgii Casp. Obere Epidermis des Blattes mit braunen Manganflecken, von oben gesehen. Vergr. etwa 300.

Fig. 4. Der Querschnitt desselben Blattes zeigt, dass die Manganflecke in der oberen Wand der Epidermis ihren Sitz haben, viel weniger in der unteren Epidermis. Vergr. 500.

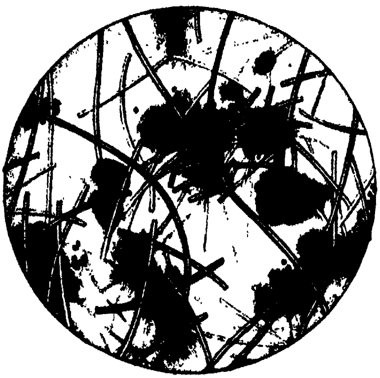
1



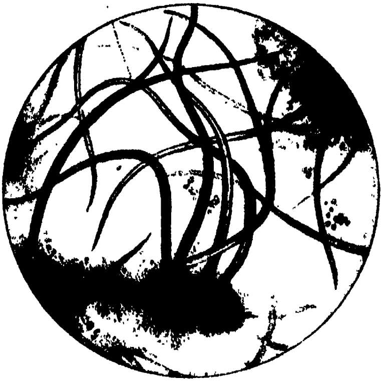
2



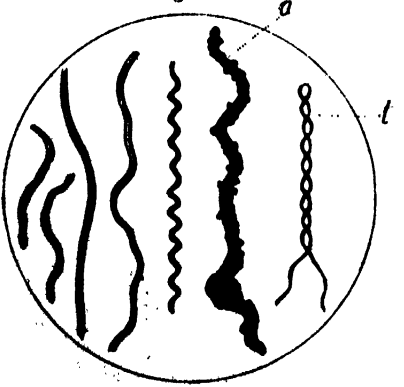
3



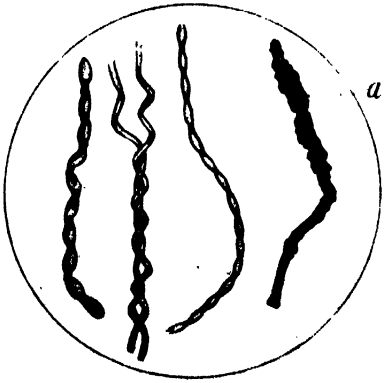
4



5



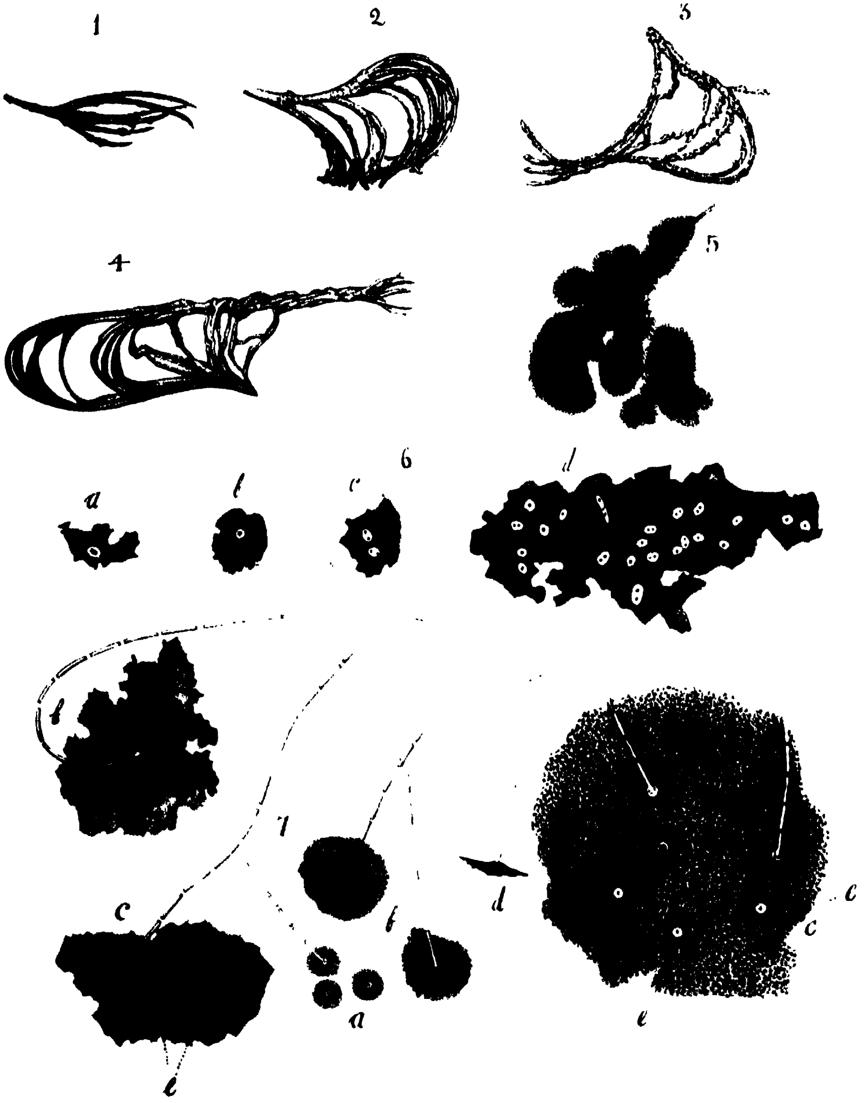
6



(B. Sakuma delin.)

EISENORGANISMEN
HANS MOLISCH.

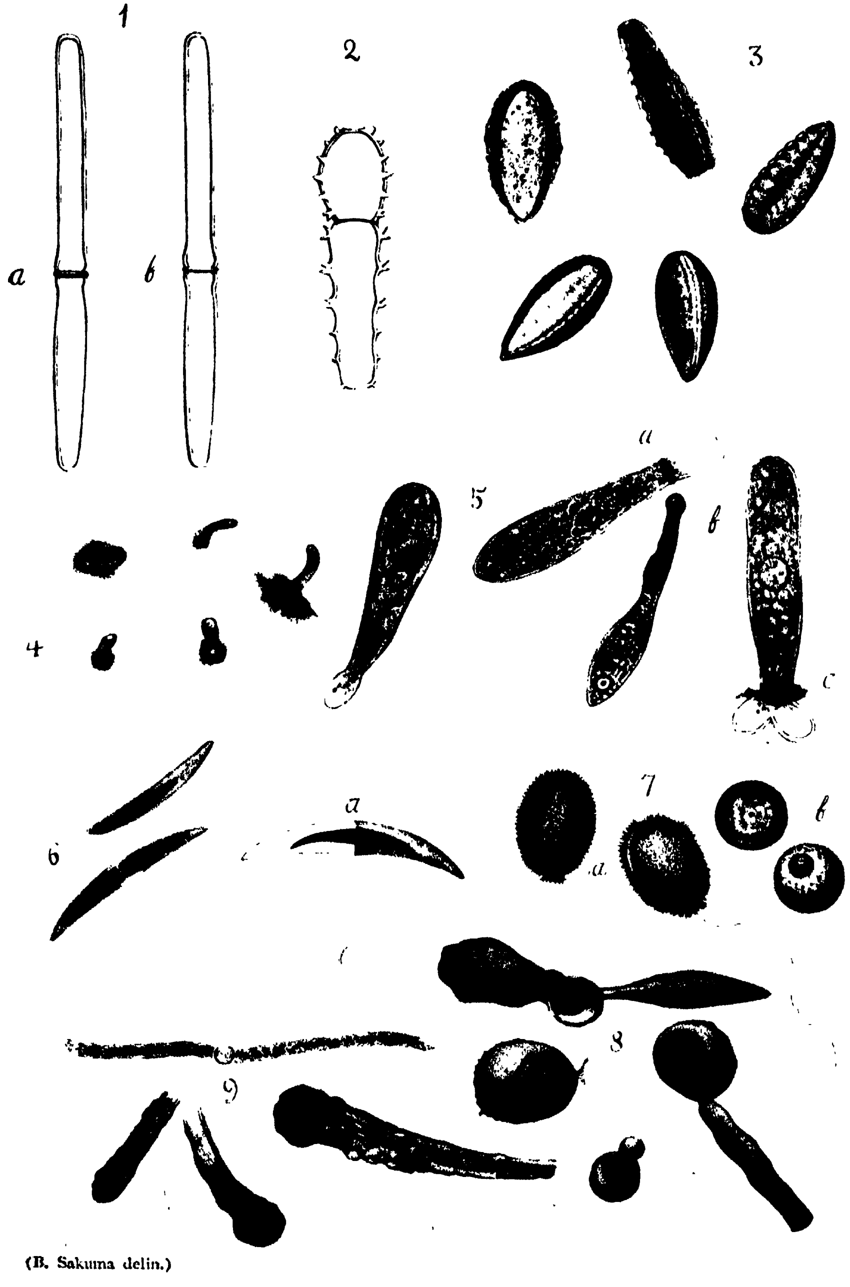
TAFEL I



(B. Sakuma delin.)

EISENORGANISMEN

HANS MOLISCH.

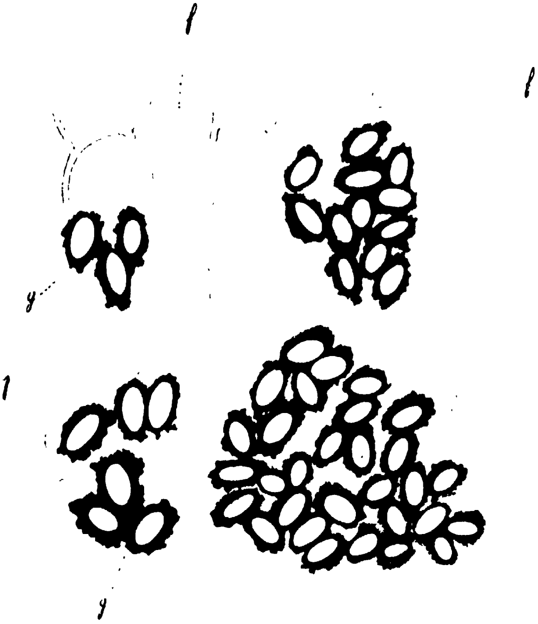


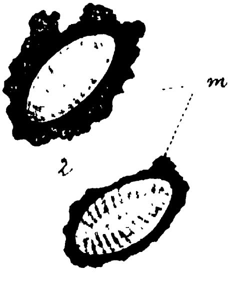
(B. Sakuma delin.)

EISENORGANISMEN

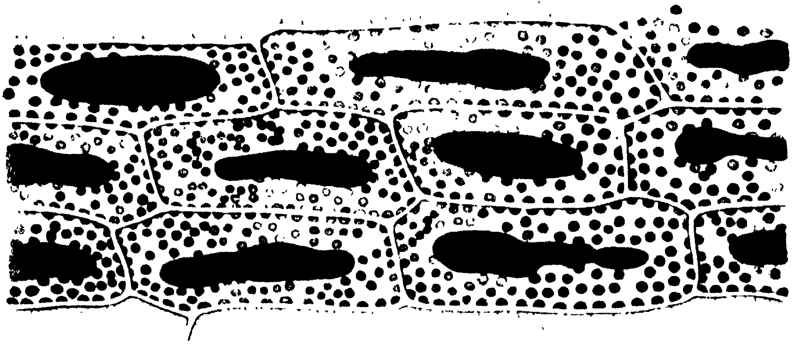
HANS MOLISCH.

1

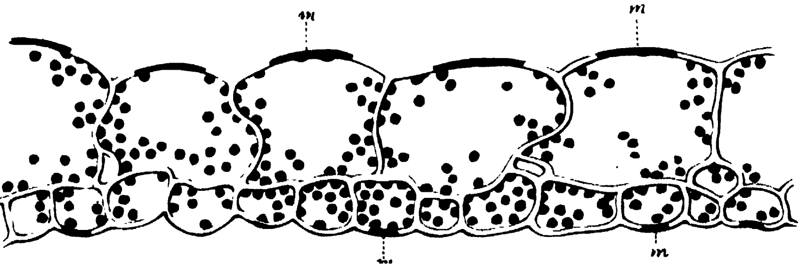




3



4



(B. Sakuma delin.)

BOTANISCHE BEOBACHTUNGEN IN JAPAN.

VON

PROF. DR. HANS MOLISCH.

IX. MITTEILUNG.

ÜBER DIE SYMBIOSE DER BEIDEN LEBERMOOSE BLASIA PUSILLA L. UND CAVICULARIA DENSA ST. MIT NOSTOC.

(MIT 2. TAFELN UND 1 TEXTFIGUR.)

A.

BLASIA PUSILLA L.

1. Historisches.

Blasia pusilla ist bekanntlich ein weit verbreitetes Lebermoos. Es findet sich in Europa, Nordamerika und Australien[1], ist aber auch in Japan keine Seltenheit. In der Umgebung von Sendai kommt es auf den abschüssigen verwitterten Tuffelsen sehr häufig vor. Ich habe Gelegenheit gehabt, während meines Aufenthaltes in Japan die Pflanze an vielen Orten zu beobachten, habe, ich kann wohl sagen, mehrere Hunderte mikroskopisch geprüft und ausnahmslos mit Nostoc inficiert gefunden.

Die Symbiose zwischen Blasia und Nostoc ist schon lange bekannt und manche wertvolle Angaben finden sich in der Literatur vor, doch gerade das Interssanteste und Wichtigste dabei, die Physiologie dieser ganzen Erscheinung harrt noch der Aufklärung. Goebel[2] hat daher ganz recht, wenn er sagt: „Wie diese Symbiose anfzufassen ist, darüber fehlen uns noch alle experimentellen Belege."

---

[1] Schiffner V., Hepaticae, in Engler-Prantl, die natürlichen Pflanzenfamilien. I. T. 3. Abt. I. Hälfte p. 57.

[2] Goebel K., Organographie der Pflanzen. 2. Aufl. 2. Th. Specielle Organographie. 1. Heft, Bryophyten. p. 656. Jena 1915.

Szymánski[1] hat gelegentlich seiner Untersuchungen über zwei neue Chlorophyceen in Lemna auch die Nostoccolonien bei Anthoceros laevis, A. punctatus und Blasia studiert, hat aber bei Blasia sein Augenmerk hauptsächlich nur auf die Beschaffenheit der verzweigten Schläuche gerichtet, die die Nostoccolonie durchsetzen.

Leitgeb[2] zeigte, dass die Nostoccolonien der Anthoceroten im wesentlichen denselben Bau haben wie die der Blattohren der Blasia und dass nur insofern ein Unterschied besteht als bei Blasia die Bildung der Schläuche von einem morphologisch bestimmten Punkte ausgeht.

Eingehender beschrieben wurde die Entstehung und die Beschaffenheit der erwähnten Schläuche bei Blasia von Waldner[3]. Er bestätigte die ältere Erfahrung, dass die Bildung der Schläuche im Blattohre von Blasia bei Nostocinfection von dem in den Hohlraum des Blattohres hineinragenden Trichome ausgeht. Dieses Haar besteht aus einer abgestutzt kegelförmigen Basalzelle und einer kopfförmigen Endzelle. Meistens entstehen die Schläuche aus der Basalzelle und bilden nie eine sondern in ihrer Gesamtheit mehrere oft vielfach verzweigte Zellen.

2. Die Nostoccolonien und die beiden Brutkörper.

Betrachtet man den Thallus mit freiem Auge oder noch besser mit der Lupe, so sieht man längs des Thallusrandes an beiden Seiten je eine Reihe von schmutzig grünen Punkten, die sich von dem hellgrünen Thallusgewebe durch ihre viel dunklere Farbe stark abheben. Fig. 1. Tafel I. Es sind grosse Nostoccolonien, die im Verein mit den Schläuchen die Blattohren ganz ausfüllen.

Einen viel klareren Einblick gewährt das Mikroskop. Man sieht dann Inseln von gewöhnlich ellipsoidischer, seltener kreisrunder Gestalt, die aus einer grossen Menge von Nostoczellen bestehen. Ihre Farbe ist nicht immer gleich. Bald sind sie gelblichbraun, bald oliven- oder spangrün. Der Durchmesser der Nostoczellen schwankt zwischen

---

[1] Szymanski, Über einige parasitische Algen. Inaug. Dissert. d. Universität Breslau 1878.

[2] Leitgeb H., Die Nostoccolonien im Thallus der Anthoceroteen. Sitzber der Kais. Akad. d. Wissensch. in Wien. 77. Bd. 1878. I. Abt. p. 411.

[3] Waldner M., Die Entstehung der Schläuche in den Nostocoolonien bei Blasia, Ebenda 78. Bd. 1879, p. 294.

4.5–6.5 μ, die Kolonie ist von den bereits erwähnten Schläuchen durchsetzt. Fig. 4, 5, Tafel I.

Wenn man die Kolonien mit conc. Schwefelsäure behandelt, so werden sie offenbar wegen ihres hohen Carotingehaltes sofort vorübergehend himmelblau.

In Eisessig werden sie zunächst bräunlich, schliesslich farblos, eine Blau- oder Violettfärbung bleibt aus, ein Zeichen, dass hier nur wenig Phycocyan vorhanden ist. Dass aber dieser Farbstoff hier dennoch vorkommt, zeigt sich nach Behandlung mit Chloralhydrat (2 Teile Wasser und 5 Teile Chloralhydrat), denn darin werden sie deutlich hellviolett.

Blasia besitzt bekanntlich zweierlei Brutköper. Die einen entstehen in besonderen, dem Sprosscheitel genäherten flaschenförmigen Behältern und die andern frei auf der Oberfläche. Gestaltlich sind sie auffallend verschieden. Die in den Behältern gebildeten Brutkörper stellen gestielte ellipsoidische Zellkörper dar, wie sie Fig. 2, Tafel I, zeigt. Die frei entstehenden sind viel grösser, unregelmässig gezackt und werden dieser ihrer Form wegen als gezackte Brutschüppchen oder „ Sternschuppen " bezeichnet. Fig. 3.

Aber nicht nur gestaltlich auch physiologisch unterscheiden sich die beiden und zwar durch 2 Eigentümlichkeiten.

1. Die runden haben eine Ruheperiode und keimen regelmässig erst am Ende des Winters und im Frühling, die gezackten aber schon im Herbst, vorausgesetzt, dass man sie von der Mutterpflanze weg auf ein geeignetes Substrat bringt z. B. auf mit gewöhnlichem Wasser getränktes Filtrierpapier oder auf Sand.

Es zeigt sich hier dasselbe wie bei den Brutkörpern von Marchantia, die, solange sie sich noch im Brutbecher befinden, gewöhnlich nicht oder schwer keimen, wohl aber, wenn sie aus diesen auf ein geeignetes Substrat gebracht werden. Mein Schüler, Herr Dr Oppenheimer[1], hat meine Ansicht über das Verhandensein von Hemmungsstoffen in den Brutbechern von Marchantia und den Früchten verschiedener Pflanzen einer genauen Prüfung unterworfen und kommt zu dem Schlusse, dass besondere in dem Brutbechern von Marchantia und den Früchten vieler

---

[1] Oppenheimer H., Das Unterbleiben der Keimung in den Behältern der Mutterpflanze. Sitzber. d. Kais. Akad. d. Wissensch. i. Wien. Mathem-naturw. Kl. Abt. I. 131. Bd. 1922, p. [illegible] 9.

Phanerogamen (Solanum Lycopersicum, Nicotiana rustica, Capsicum annuum, Cucumis sativa etc) vorhandene Hemmungsstoffe die Keimung in den Behältern der Mutterpflanze hintanhalten.

2. Die runden Brutkörper sind mit der Nostocalge nicht inficiert, die gezackten aber fast immer. Fig. 2 u. 3, Taf. I.

Die „ Sternschuppen " bestehen aus einem gezackten Gewebekörper, in dem auch schon die sogenannten Blattohren entwickelt sind. Ausserdem bemerkt man schon in Wasserpraeparaten, noch deutlicher in Chloralhydratlösung 1 oder 2 runde Behälter (Blattohren), die mit Nostoc erfüllt sind. Fig. 3 Taf. I.

Nach längerer Einwirkung des Chloralhydrats nehmen die Algen in Folge ihres Phycocyangehaltes eine hellviolette Farbe an, während sie im natürlichen Zustande schmutzig bräunlich oder spangrün erscheinen. Sie stechen dann von dem benachbarten Chlorophyllparenchym ab und werden dadurch ungemein deutlich.

Fast jede Schuppe enthält 1-2 solcher scharf begrenzter Algeninseln. Doch kann es auch häufig vorkommen, dass sich Nostoc auch frei an der Oberfläche der gezackten Brutkörper vorfindet, was ja begreiflich ist, da ja die Alge von aussen in das Blattohr einwandert. Die Fig. 7, Taf. I. zeigt solche freie „ epiphytische " Nostoccolonien.

Vielleicht üben die in den Blattohren sich entwickelnden schlauchartigen Haare eine chemotaktische Wirkung auf die in der Umgebung befindlichen Nostoczellen aus, so dass sie den Weg in die Behälter finden.

3. Die Ablagerung von kohlensaurem Kalk im Thallus.

Bevor ich zu den physiologischen Versuchen übergehe, will ich noch einer auffallenden Erscheinung gedenken, die ich im Thallus von Blasia und, wie später noch berichtet werden wird, auch bei Cavicularia beobachtet habe: die oft mehr oder minder starke Ablagerung von CO_3Ca. Man sieht schon bei schwacher Vergrösserung parallel zur Längsachse des Thallus 1, 2 oder 3 dunkle Streifen, die bald auf eine längere bald auf eine kürzere Strecke dahinziehen. Fig. 8, s, Taf. I. Bei stärkerer Vergrösserung kann man erkennen, dass diese Streifen aus mehr oder minder grossen Stücken einer weissen kristallinischen Masse bestehen, die etwas in die Länge gestreckte, zu einer Art Bündel vereinigte Parenchymzellen ausfüllen. Oft so vollends, dass dadurch genaue Abgüsse der betreffenden Zellen entstehen. Durch dass Messer oder durch den Druck mit dem Deckglas werden sie oft zertrümmert und bilden dann ein Haufwerk von unregelmässigen Bruchstücken. Fig. 9. Taf. I.

In vielen Individuen sind die Kalkstreifen sehr häufig, in manchen spärlich oder gar nicht vorhanden.

Die Kalkmassen leuchten in Polarisationsmikroskop deutlich auf, lösen sich in Salzsäure, Essigsäure und mehreren anderen organischen und anorganischen Säuren unter Aufbrausen und in Schwefelsäure unter gleichzeitiger Bildung von Gipsnadeln auf.

Auf Querschnitten durch den Thallus sieht man, dass die kalkführenden Zellen Bündel bilden, die den Thallus der Länge nach durchziehen. Die Bündel sind im Durchmesser 4 Zellen breit und diese haben etwas dickere, lichtbräunliche Wände, wodurch sie sich von den benachbarten gewöhnlichen Parenchymzellen etwas abheben.

4. Die Reinkultur der Blasia-Nostoc.

Zunächt interessierte mich die Frage, ob es möglich ist, die Nostocalge für sich, also unabhängig von dem Lebermoos in Reinkultur zu ziehen. Zur besseren Beurteilung des gegenseitigen Verhältnisses der beiden Symbionten erscheint die Lösung dieser Frage nicht nur wünschenswert, sondern sogar notwendig.

Um zur Reinkultur zu gelangen, habe ich zunächst ein möglichst reines, mit Nostoc inficiertes Thallusstück im destillierten Wasser mehrmals gereinigt und dann mit sterilisierten Nadeln die Kolonie freigelegt also vom Lebermoos vollständig getrennt. Eine solche Kolonie wurde dann in eine Agarlösung von folgender Zusammensetzung

1000 g	H_2O
¼ g	KNO_3
¼ g	$MgSO_4$
¼ g	K_3PO_4
Spur	$FeCl_3$
18 g	Agar

eingeimpft und dann an einem Nordfenster unter Glassturz, um Staub abzuhalten und rasches Austrocknen zu verhindern, bei gewöhnlicher Zimmertemperatur aufgestellt. Schon nach 4 Wochen waren dunkel spangrüne Kolonien entwickelt. Von diesen wurden, da neben den

Nostoccolonien auch Bacterien- und Chlorphyceen-Kolonien auftraten, neuerdinge abgeimpft und so schliesslich tadellose Reinkulturen erhalten. Diese ziehe ich seit 2 Jahren sowohl in Petrischalen als auch in Proberöhren auf Agar mit bestem Erfolge weiter. Eine Reinkultur ist in der Fig. 6 abgebildet.

Es ist wohl darauf zu achten, dass die Nostoccolonien wirklich rein d. h. frei von Bakterien sind. Es ist das eine sehr schwierige Aufgabe, aber nach vielen Versuchen habe ich sie doch gelöst.

Die Alge der Blasia-Kolonie und der Reinkultur stimmen im Aussehen und der Grösse überein, auch bezüglich der Grenzzellen. Die Kolonien der Reinkultur erscheinen, wenn klein, olivenfarbig, wenn gross, fast schwarzgrün. Während der letzten 2 Jahre habe ich, indem ich von Zeit zu Zeit abimpfte und neue Kulturen anlegte, die Reinkultur in vielen Generationen erneuert und mich auf diese Weise überzeugt, dass die Blasia-Nostoc auch ohne Wirt ausgezeichnet gedeiht.

So möchte es auf den ersten Blick scheinen, als ob die Alge von dem Zusammenleben mit dem Lebermoos nichts hätte. Doch mich dünkt, dass man mit solchen Schlüssen vorsichtig sein muss, ich will mich aber erst darüber äussern, wenn ich die betreffenden Verhältnisse auch für Cavicularia geschildert habe.

5. Versuche, nostocfreie Blasia und Cavicularia zu ziehen.

Für die Beurteilung des Symbiontenverhältnisses wäre es auch wichtig zu wissen, ob das Lebermoos auch ohne die Alge gedeihen kann.

In der Natur habe ich Blasia und wie ich gleich hinzufügen will, auch Cavicularia nie ohne Nostoccolonien im Thallus gefunden. Alle untersuchten Exemplare — und ich habe Hunderte von verschiedenen Standorten geprüft — waren ohne Ausnahme inficiert.

Ich versuchte daher durch Kultur zum Ziele zu gelangen. Ich säete am 1. Mai 1923 auf sterilen Sand in Petrischalen aus.

a) Dauerbrutkörper von Blasia.
b) Sternschuppen von Blasia.
c) Sternschuppen von Cavicularia.

Alle keimten, entwickelten kräftige, jedoch, weil in dunst. gesättigtem Raume, „verspindelte" Thallome; waren aber, als ich sie am 30. Juli 1923 untersuchte, mit Nostockolonien durchwegs inficiert.

Bei Wiederholung des Versuchs ergab sich leider dasselbe Resultat. Da ich die Petrischalen als auch den Sand nach allen Regeln bakteriologischer Technik sterilisierte, die Kulturen unter Glasglocken vor Staub schützte und nur mit steriler Nährlösung begoss, so muss ich annehmen, dass an der Oberfläche der Brutkörper sich stets Nostoczellen vorfinden und dass es daher nicht möglich ist, sich davon unabhängig zu machen.

Ich kam dann auf den Gedanken, kleine anscheinend noch nicht inficierte Teile des Thallus zur Fortpflanzung gewissermassen als Stecklinge zu verwenden, jedoch, wie ich gleich bemerken will, gleichfalls mit negativem Resultat. Die Stecklinge giengen entweder zugrunde oder waren, wenn sie sich weiter entwickelten, gleichfalls mit Nostockolonien versehen, offenbar, weil auch sie schon mit einzelnen Nostoczellen am Beginne des Versuchs oberflächlich inficiert waren.

Kurz gesagt: es war nicht möglich, trotz aller darauf verwendeten Mühe nicht inficierte Blasia und Cavicularia zu erhalten.

Obwohl das Ergebnis ein negatives ist, so ist es doch nicht ohne Wert, weil es zeigt, dass es dem Lebermoos gar nicht gelingt, sich von Nostoc loszumachen, weder in der Natur noch in der Kultur.

Hingegen gedeiht, wie bereits von mir gezeigt wurde, die Alge sowohl in dem Wirte als auch ausserhalb, unabhängig von ihm.

Nicht unerwähnt will ich lassen, dass die Nostocalge sich in einzelnen Gefässen auch auf dem Sande entwickelte und dies ist gleichfalls ein Beweis, dass man in das sterile Kulturbett mit den Brutkörpern Nostoczellen einführt, die oberflächlich anhängen.

6. Kann die Blasia-Nostoc freien Stickstoff assimilieren?

Zur Klärung des Symbiontenverhältnisses legte ich mir auch die Frage vor, ob der Algensymbiont gebundenen Stickstoff in Form von Nitraten oder Ammoniaksalzen durchaus benötigt oder ob er vielleicht ähnlich wie gewisse Bakterien im Stande ist, den freien Stickstoff der Atmosphäre zu assimilieren.

Um diese Frage zu beantworten, machte ich die folgenden Versuche. Ich bereitete zweierlei Agarlösungen A und B, die sich nur dadurch von einander unterschieden, dass die eine ein Stickstoffsalz enthielt, die andere aber nicht. Die Zusammensetzung der Lösungen war wie folgt:

A (ohne N)	B (mit N)
1000 g H_2O	1000 g H_2O
¼ g $MgSO_4$	¼ g $MgSO_4$
¼ g K_3PO_4	¼ g K_3PO_4
	¼ g KNO_3
Spur $FeCl_3$	Spur. $FeCl_3$
18 g Agar	18 g Agar

Für die Herstellung der beiden Nährlösungen wurden staubfreies dest. Wasser und sehr reine Nährsalze (purissimum pro analysi) verwendet, um etwaige Spuren von Stickstoffverbindungen auf ein Minimum herabzudrücken.

Je 5 Eprouvetten wurden mit je einer der beiden Lösungen gefüllt, mit einer Spur einer Reinkultur geimpft und dann an einem Nordfenster unter gut abschliessendem Glassturz aufgestellt. Unter einem Glassturz, um die Verdampfung des Wassers aus dem Nährmedium möglichst zu verringern und irgendwelche Dämpfe von Stickstoffverbindungen, wie sie sich in der Atmosphaere spurenweise vorfinden, auszuschalten.

Der Versuch wurde am 27. IX 1923 begonnen.

Am. 20. X. 1923 also nach 23 Tagen war in beiden Nährmedien gute Entwicklung zu bemerken.

Am. 1. XII. 1923 war die Entwicklung weiter vorgeschritten.

Am. 23. I. 1924 war die Oberfläche des Agarmediums mit einer dicken Lage von schwärzlich grünem Nostoc bedeckt. Auch in der Tiefe des Agar bis zu 2 cm herab waren Kolonien vorhanden aber nur kleine. Ein Unterschied in der Schnelligkeit der Entwicklung und der Menge der producierten Alge war in dem N-hältigen und N-freien medium nicht zu bemerken.

Der Versuch wurde 3 mal wiederholt und gab im wesentlichen dasselbe Resultat. Wohl wissend, dass Algen oft mit wenig mineralischer Substanz auskommen, machte ich mir den Einwand, dass das verwendete Agar vielleicht doch genügend gebundenen N enthalten hat, um die Entwicklung des Nostoc zu ermöglichen. Es wurde daher noch ein Versuch mit lange in fliessendem Leitungswasser und dann mit dest. Wasser gewaschenem Agar gemacht aber wieder mit demselben Ergebniss wie vorhin. Nun war aber noch der Einwand möglich, dass in dem Agar auch organische, im Wasser unlösliche N-Verbindungen vorhanden sind, die vielleicht von der Alge assimiliert werden könnten. Um auch diesen eventuellen Fehler zu beseitigen, machte ich mich vom Agar ganz unabhängig und stellte dieselben Versuche mit Nährlösungen ohne Agar an. Die Zusammensetzung der beiden Nährlösungen mit und ohne Stickstoff war ganz die gleiche, wie früher nur war das Agar weggelassen.

Beginn des Versuchs am 23. IX. 1923 mit je 5 Erlenmayerkolben zu je 50 cm^3 der Lösungen, geimpft mit einer Reinkultur von Blasia-Nostoc.

Am. 20. X. 1923 war in beiden Nährlösungen gute Entwicklung. in 2 N-Kolben anscheinend bessere Entwicklung zu beobachten.

Am 23. I. 1924 war in allen Kolben sehr gute Entwicklung in Form von Häuten an der Oberfläche der Flüssigkeit und des Glases festzustellen, auch in der Menge konnte man keinen Unterschied in den N-freien und N-hältigen Lösungen konstatieren.

Um noch dem Einwand zu begegnen, dass die Nostoc-Alge in der N-freien Nährlösung wuchs, weil vielleicht kleine Mengen von gebundenem N in Form von Ammoniak aus der atmosphärischen Luft in die Lösungen hineinkommen und von der Alge assimiliert wurden, machte ich noch folgende Versuche.

14 Erlenmayerkolben wurden mit Nährlösung zu je hundert cm^3 beschickt, 7 davon mit N-hältiger, 7 mit N-freier Lösung von folgender Zusammensetzung.

Mit N	ohne N.
1000 g H_2O	1000 g H_2O
$\frac{1}{4}$ g SO_4Mg	$\frac{1}{4}$ g SO_4Mg
$\frac{1}{4}$ g NO_3K	$\frac{1}{4}$ g K_3PO_4
$\frac{1}{4}$ g K_3PO_4	
Spur $FeCl_3$	Spur $FeCl_3$

Hierauf wurden alle Kolben sterilisiert und nach dem Abkühlen mit bakterienfreier Reinkultur von Nostoc der Blasia geimpft.

Die N-freien Kolben wurden unter grossen Glasglocken, die auf mattgeschliffenen Gasplatten luftdicht aufgestellt waren, untergebracht. Durch diese Glocken wurde täglich $\frac{1}{2}$ Stunde lang Luft geführt, die aber vorher durch Schwefelsäure und damit getränkten Bimstein geleitet wurde, um etwaige Spuren von N-Verbindungen der Luft zurükzuhalten. Da die Schwefelsäure die Luft zu sehr trocknet und zu starke Verdampfung der Nährlösung veranlassen würde, wurde zwischen dem Schwefelsäuregefäss und dem Algenversuchsgefäss noch eine Waschflasche mit dest. Wasser eingeschaltet.

Die andern N-hältigen Kolben dienten als Kontrolle, standen daneben unter einer Glasglocke, vor Staub geschützt, doch so, dass die Luft unter dem Glockenrand ein- und austreten konnte.

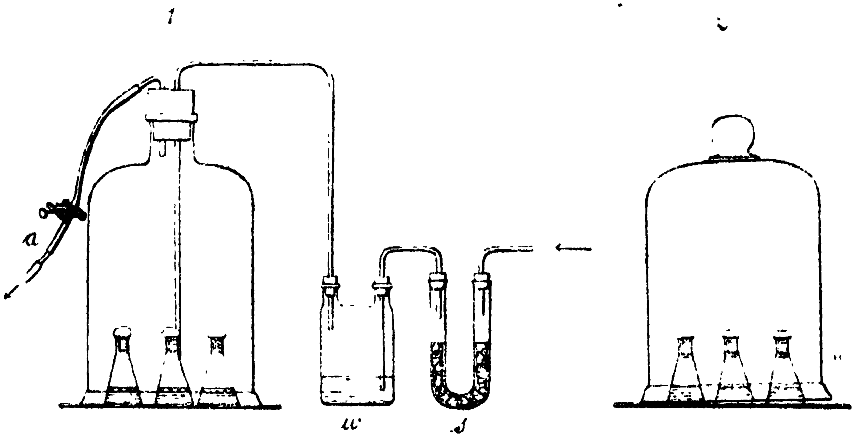


Fig. Versuchsanstellung über die Kultur von Blasia- und Cavicularia-Nostoc ohne (1) und mit gebundenem N (2).

1. Kulturraum, durch den Luft, befreit von NH_3 geleitet wurde. Die Luft passierte zuerst das mit Bimstein und Schwefelsäure gefüllte U-Rohr s, dann die Waschflasche w und trat dann in die mit den Kulturgläsern beschickte Glasglocke. Die Luft wurde von a aus angesaugt.
2. Kontrollgefäss mit Kulturgläsern, enthaltend gebundenen N.

Die Kolben standen in einem grossen geräumigen Saal an einem Nordfenster, günstigem diffusen Lichte ausgesetzt.

Der Versuch begann am 28. IV. 1924 und wurde am 13. IX. 1924 abgeschlossen. In beiden Gefässen sowohl mit als auch ohne N wuchs Nostoc, aber in den Kolben ohne N auffallend besser, hier waren fingerdicke Rasen. Der Unterschied war in die Augen springend wie schon der Augenschein noch besser aber die Gewichtsbestimmung lehrte. Schon nach etwa 4 Wochen war der Unterschied merklich und zu dieser Zeit war die in der N-freien Kultur vorhandene

Alge tief grün, die in N-hältiger Lösung mehr bleich. Am Schlusse des Versuchs war die Nostoc-Alge in der N-Lösung an der Oberfläche der Rasen bräunlichgrün, tiefer bleich, in der N-freien Kultur oben deutlich grün, im übrigen auch bleich. Schliesslich wurde das Erntegewicht für beide Kulturen bestimmt.

Trockengewicht der Nostoc-Ernte.

N-freie Lösung		N-hältige Lösung	
Kolben Nr.		Kolben Nr.	
1	0.0290 g	1	0.0095 g
2	0.0255	2	0.0070
3	0.0445	3	0.0040
4	0.0250	4	0.0105
5	0.0215	5	0.0105
6	0.0345	6	0.0090
7	0.0300	7	0.0065
Summa	0.2100 g	Summa	0.0570 g

Das Erntegewicht der Kulturen ohne gebundenen N verhält sich daher zu dem der Kulturen mit gebundenem N wie 210 : 57 oder 3.6 : 1.

Das Überraschende in diesem Versuche war, dass die Nostoc sich in den salpeterfreien Gefässen, die in filtrierter Luft standen, sich viel reichlicher entwickelten als in den nitrathältigen, die sich in gewöhnlicher unfiltrierter Luft entwickelten.

Um dieses auffallende Ergebnis noch mehr zu stützen und um eine etwa vorhandene Fehlerquelle auszuschliessen, wurde der eben geschilderte Versuch nochmals in derselben Weise, nur mit dem Unterschiede wiederholt, dass ich den Nährlösungen noch 0.025% $CaCl_2$ hinzufügte.

Beginn des Versuchs am 16. IX 1924.

Am 24. X. 1924 war die Entwicklung in den Kolben mit Salpeter mässig, ohne Salpeter ausgezeichnet.

Am 4. XII. 1924 waren die Kulturen in allen Kolben ohne Salpeter üppig, mit Salpeter etwas geringer.

Der Versuch wurde am 17. XII 1924 abgeschlossen. In den Gefässen ohne gebundenen N war die Entwicklung anfangs auffallend stärker als in den Kontrollgefässen.

Trockengewicht der Nostocernte.

N-freie Lösung		N-haltige Lösung.	
Nro. d. Kolbens		Nro. d. Kolbens.	
1	0.0235 g	1	0.0170 g
2	0.0170	2	0.0245
3	0.0200	3	0.0155
4	0.0195	4	0.0330
5	0.0240	5	0.0150
6	0.0200	6	0.0200
	0.1240 g		0.1250 g

Die Erntegewichte waren am Ende des Versuchs fast gleich.

Aus diesen Versuchen in ihrer Gesamtheit glaube ich den Schluss ziehen zu dürfen, *dass die Blasia-Nostoc imstande ist, den freien Stickstoff der Atmosphäre zu assimilieren und auch ohne gebundenen Stickstoff das Auslangen zu finden.*

Dieser Befund erscheint von Interesse, weil bisher meines Wissens noch für keine Cyanophycee die Fähigkeit der Assimilation freien N erwiesen wurde und daher wäre es empfehlenswert, verschiedene Cyanophyceen daraufhin zu prüfen. Es wäre ja möglich, dass die erwähnte Eigenschaft unter den Blaualgen verbreiteter und nicht bloss auf die Blasia-Nostoc und die damit identische Cavicularia-Nostoc beschränkt ist.

Die in den beiden Lebermoosen lebende Nostoc würde damit in Gegensatz stehen zu den von mir seinerzeit geprüften Chlorophyceen, Microthamnion Kützingianum Näg. a genuinum (Näg.) Hansgirg, Stichococcus baccilaris major Rbh., Ulothrix subtilis (?) Kg. und Protococcus, die ohne gebundenen N nicht gedeihen.[1]

Welchen Nutzen die Nostoc durch die Fähigkeit der Assimilation des freien Stickstoffs der Blasia gewährt, soll am Ende der Abhandlung, nachdem die entsprechenden Verhältnisse für Cavicularia behandelt worden sind, erörtert werden.

---

[1] Molisch H., Die Ernährung der Algen. (Süsswasseralgen) I. Abhandlung. Sitzber. der Kais. Akad. d. Wissensch. in Wien, Bd. CIV. Abt. 1. 1895 p. 793 u 795.

B.

CAVICULARIA DENSA ST.

1. Vorkommen und Brutkörper.

Am 24 Dezember 1922 sammelte ich in Sendai auf tuffigem feuchten Gestein ein mir unbekanntes Lebermoos, das ich als Cavicularia densa St. bestimmte. Seither habe ich in der Umgebung der genannten Stadt mehrere Standorte kennengelernt und das Moos stets ohne Ausnahme mit Nostoc inficiert gefunden.

Die Bestimmung dieses interessanten Lebermooses machte anfangs einige Schwierigkeit, da es in die mir hier zur Verfügung stehenden Bestimmungschlüssel noch nicht aufgenommen war. Als ich aber die Beschreibung der Cavicularia bei Goebel[1] las, war ich sicher, dass ich es mit dieser Gattung, die bisher nur in Japan gefunden wurde, zu tun hatte.

Der Thallus ist ziemlich gross, etwa von den Dimensionen einer mittelgrossen Fegattella conica, dichotom verzweigt, nicht gefeldert und am Rande ziemlich gewellt. Cavicularia habe ich oft zusammen mit Blasia pusilla, Solenostoma grandistipula und Conocephalus supradecompositus auf verwitterten Tuffelsen mit verticalen oder etwas schiefen Wänden gefunden. Standorte, wie sie Marchantia, Lunularia und Makinoa lieben, die sich auf mehr ebenem, nährstoffreichen Boden wohlfühlen, scheint Cavicularia zu meiden.

Zwischen der recht dicken Thallusmitte und dem Thallusrande findet sich beiderseits eine Reihe von einander entfernten dunklen Punkten, die entweder rund oder parallel zur Längsachse des betreffenden Thallusastes in die Länge gezogen erscheinen. Diese Punkte stellen die mit dem Lebermoos in Symbiose vereinigten Nostockolonien dar, Tafel II. Fig. 1 n.

Es ist nicht schwer, mit Nadeln eine Kolonie aus dem Thallus zu isolieren und dann für sich allein bei stärkerer Vergrösserung zu betrachten. Sie besteht aus der Alge und schlauchartigen Haaren, die die Kolonie mit ihren zahlreichen Verzweigungen nach verschiedenen Richtungen durchsetzen.

---

[1] Goebel K., Organographie d. Pflanzen. 2. Teil. Spezielle Organographie, 1. Heft. Bryophyten, p. 669.

Die Haare sind farblos, einzellig, oft verzweigt, enden stumpf, oft keulenförmig, enthalten reichlich Plasma, manchmal Spuren von Stärke und strahlen radiär von einem Zentrum aus. Fig. 5. h.

Die Nostoczellen sind spangrün und haben einen Durchmesser von 3.5–6.8 μ. Einen Unterschied zwischen der Blasia- und der Cavicularia-Nostoc konnte ich nicht bemerken.

Wie bereits Schiffner[1] gefunden und Goebel[2] bestätigt hat, treten auch bei Cavicularia zweierlei Brutkörper auf. Sie entstehen in dorsalen, unregelmässig halbmondförmigen offenen Behältern, die gestaltlich von den flaschenförmigen der Blasia ganz und gar abweichen. Fig. 2 b. Die einen Brutkörper ähneln sehr den „Sternschuppen" der Blasia — Fig. 3. — die andern — Goebel nennt sie Dauerbrut-Körper — sind kleiner, rundlich aber mit vorspringenden, kurzen kegeligen Zellen an der Oberfläche. Bei passender Lage kann man deutlich die Stielzelle oder ihre meist bräunlich gefärbte Ansatzstelle — Fig. 4 s — und etwa im Aequator rechts und links je eine einspringende Stelle sehen, die die Initialen bergen. Fig. 4. i.

Solange die Sternschuppen in den Brutbehältern liegen, bilden sie in der Regel keine Wurzelhaare, jedoch schon nach 3–4 Tagen, wenn man sie aus den Behältern in gewöhnliches Leitungswasser bringt. Auch hier dürften wohl Hemmungsstoffe, die in den Brutbehältern abgeschieden werden, die Keimung auf der Mutterpflanze verhindern, Ich machte die Versuche in Dezember und Jänner.

Die zweite Art der Brutkörper, die kleineren mit den dicken Wänden — Fig. 4 — keimen im Jänner noch nicht, weil sie eine Ruheperiode haben im Gegensatz zu den andern.

Beide Arten von Brutkörpern finden sich zusammen in dem Brutbehälter, es machte aber den Eindruck auf mich, dass die Dauerbrutkörper bei sehr feucht gehaltenen Kulturen zurücktreten oder fehlen, während sie bei sehr trockenen Kulturen in grosser Zahl vorhanden sind.

Von grossem Interesse ist es nun, dass schon die Sternschuppen sehr häufig mit Nostoc inficiert sind, während die Dauerbrutkörper vor der Keimung keine Infection zeigen.

Die Sternschuppen haben häufig 1 oder 2, die grossen sogar mitunter 3 u. 4 Nostocherde—Fig. 3 n n,—, die besonders in Chloralhydrat ungemein deutlich erscheinen, zumal sie darin eine violette Farbe annehmen und dadurch von dem benachbarten grünen Parenchym abstechen. Die violette Farbe rührt vom Phycocyan der Alge her. Das Chlorophyll wird zerstört, gelöst und dadurch kommt der früher durch Blattgrün verschleierte violette Farbstoff zum Vorschein.

---

[1] Schiffner V., Österr. botan. Zeitschrift 1899, p. 392.

[2] Goebel K., l. c. p. 669–670.

Aus dem Gesagten geht schon hervor, dass Cavicularia morphologisch und biologisch Blasia nahe steht und ich kann hinzufügen, dass sich auch die massenhafte Anhäufung von kohlensaurem Kalk, wie ich sie für Blasia beschrieben habe, auch bei Cavicularia eine sehr häufige, ja man kann sagen regelmässige Erscheinung ist.

2. Die Ablagerung von kohlensaurem Kalk im Thallus.

Der Querschnitt durch den Thallus von Cavicularia lässt erkennen, dass er in der Mitte und um diese herum aus einem vielschichtigen (oft bis 20 Zellagen) Parenchym besteht, das gegen die beiden Ränder rechts und links allmählig in ein wenigschichtiges übergeht. Die obersten 2 Lagen sind chlorophyllhaltig und daher grün, die tieferen farblos. Die dem Substrate anliegende „Epidermis" ist braun und liefert die Rhizoiden Fig. 7 r, die gleich darüber liegende Schichte aber ist grün. An den dünneren Rändern ist auch die unterste Zellage grün.

In dem Parenchym eingebettet finden sich ebenso wie bei Blasia Bündel von polygonalen Parenchymzellen — bei Cavicularia bis 6 Bündel an Zahl am Querschnitt — und diese sind es, die mit CO_3Ca entweder teilweise oder völlig auf weite Strecken ausgefüllt sind. Fig. 7 p.

Meines Wissens wurde eine solche ausgiebige Ablagerung von Kalkkarbonat, wie ich sie eben für Blasia und Cavicularia beschrieben habe, bei andern Lebermoosen noch nicht beobachtet. In der Fig. 8 sind einige von den Kalkausfüllungen aus den Zellen isoliert abgebildet und einzelne geben sich noch als deutliche Abgüsse der betreffenden Parenchymzellen zu erkennen.

3. Die Reinkultur der Cavicularia-Nostoc.

Ich kann mich kurz fassen, denn derselbe Vorgang, wie ich ihn für die Reinkultur der Blasia-Nostoc geschildert habe, wurde auch hier eingehalten und führte alsbald zu dem erwünschten Ergebnis. Die mit sterilen Nadeln isolierten Kolonien wurden auf einem sterilen Objektträger in einem sterilen Wassertropfen mit einer Nadel zerteilt und davon wurde in Agareprouvetten abgeimpft. Das Agar hatte dieselbe Zusammensetzung wie bei den entsprechenden Lösungen für die Blasia-Nostoc. Ich bemerke jedoch, dass man auch sehr gutes Wachstum in einem Agar mit gewöhnlichem Leitungswasser erhält. Die geimpften Agarlösungen wurde in 10 Petrischalen ausgegossen und diese, mit Glasstürzen vor Staub geschützt, an einem Nordfenster bei gewöhnlicher Zimmertemperatur aufgestellt.

Der Versuch begann am 30. März 1923. Nach 4 Wochen hatten sich bereits viele schwärzlich grüne Kolonien von Nostoc entwickelt, in manchen Schalen nur diese, in anderen auch solche von Chlorophyceen und Bakterien. Durch wiederholtes Abimpfen erhielt ich schliesslich tadellose Reinkulturen, frei von Bakterien, Grünalgen und Flagellaten. Schwierigkeiten machte insbesondere ein kleiner farbloser Flagellat mit je einer relativ grossen Geissel an den beiden Polen.

Solche Reinkulturen wachsen in Strichkulturen zwar langsam aber in 1-4 Monaten zu ansehnlichen Kolonien heran, die oft eine Dicke von 1-3 mm erreichen.

Die reine Cavicularia-Nostoc stimmt in allen Eigenschaften mit der in dem Thallus überein, es besteht nur der unwesentliche Unterschied, dass die Reinkultur die Anordnung der Zellen in Perlschnüren viel praegnanter zeigte.

Da es leicht gelang, die rein gezüchtete Nostoc immer wieder vom Neuen auf geeignetem Substrat zur üppigen Entwicklung zu bringen, so muss auch hier der Schluss gezogen werden, dass die mit Cavicularia symbiotisch vereinigte Nostoc auch ohne den Wirt in ausgiebiger Weise wächst, genau wie die Blasia-Nostoc.

4. Über die Assimilation des freien Stickstoffs durch die Nostoc der Cavicularia.

In ganz derselben Weise wie ich dies mit der Blasia-Nostoc gemacht habe, (siehe p.) wurden auch mit Reinkulturen der Cavicularia-Nostoc auf gewässertem Agar mit und ohne gebundenen N Versuche gemacht und, wie ich gleich bemerken will, mit demselben

Ergebnis. Die Alge wuchs auf dem N freien Nährboden wie auf dem N hältigen und zwar auf beiden üppig.

Um dem berechtigten Einwand zu begegnen, dass das Agar, auch wenn es lange in strömendem Wasser gewässert und mit destilliertem Wasser gewaschen wurde, doch noch gebundenen N enthalten dürfte, wurden auch Versuche mit denselben Agar freien Nährlösungen wie auf p. 177 mit Blasia gemacht.

Die eine enthielt N in Form von KNO_3, die andere war frei davon.

Auch in diesen Lösungen mit und ohne N entwickelte sich die Alge gleich gut.

Die Kolben standen unter grossen Glasglocken mit abgeschliffenem basalen Rand, um Spuren von N-Verbindungen wie sie durch die Atmosphäre oder die Zimmerluft gegeben sind, möglichst abzuhalten.

Schliesslich wurde der exakte Versuch, der auf p. 177 mit Blasia-Nostoc geschildert wurde, und in dem die Luft von Spuren von gebundenem NH_3 durch Filtration in Schwefelsäure befreit wurde, auch in ganz gleicher Weise mit bakterienfreien Reinkulturen von Cavicularia-Nostoc durchgeführt und zwar mit demselben Erfolg.

Die Nostoc-Alge wuchs mit und ohne gebundenem N, in letzterem Falle aber viel besser. Die Erntegewichte verhalten sich wie 27 : 16.

Trockengewicht der Nostoc-Ernte.

N-freie Losung		N-hältige Lösung.	
Kolben Nro.		Kolben Nro.	
1	0.0255 g	1	0.0185 g
2	0.0245	2	0.0260
3	0.0280	3	0.0160
4	0.0240	4	0.0110
5	0.0260	5	0.0160
6	0.0340	6	0.0140
	0.1620		0.1015

5. Über die Beziehungen der beiden Symbionten.

Auf Grund meiner mitgeteilten Versuche und Beobachtungen dürfte das Verhältnis zwischen Lebermoos und Nostoc folgendes sein.

1. Die Nostoc-Alge findet in dem Lebermoos einen willkommenen Schutz gegen Eintroknung. Ich habe schon hervorgehoben, dass sowohl Blasia als auch Cavicularia auf Standorten

vorkommen, wo sie mehr oder minder langen Trockenperioden ausgesetzt sind. Sie finden sich hier in Japan mit Vorliebe auf verwitterten sandigen Tuffen, die stark austrocknen können. Regenarme Zeiten würden das Gedeihen von Nostoc sehr beeinträchtigen, daher wandern sie in den Thallus der beiden Lebermoose ein und schaffen sich so einen feuchten, vor weitgehendem Austrocknen geschützten Raum.

Da die Nostoc-Alge im Thallus durch das Gewebe des Wirtes eingeschlossen ist, so muss sie auch die erforderlichen Nährsalze vom Moose beziehen. Der Wirt nimmt die Kohlensäure, das Wasser und die darin gelösten Nährsalze auf und überlässt einen Teil davon seinem Gast.

Ob die Alge noch einen andern Nutzen von dem Wirte hat, ist schwer sagen, scheint mir aber nicht sehr wahrscheinlich, da sie ja auch für sich allein also ganz unabhängig von dem Lebermoos zu gedeihen vermag.

2. Die beiden Lebermoose leben, soweit ich beobachten konnte, auf magerem stickstoffarmen Boden. Ihre Nostoc vermag freien atmosphärischen N zu assimilieren. Es scheint daher die Annahme sehr naheliegend, dass das Lebermoos die Alge so gastlich aufnimmt, weil diese ihr gebundenen N, den sie zu bereiten vermag, als Gegenleistung überlässt.

3. Eine sehr auffallende Erscheinung in den Nostockolonien sind die nie fehlenden, die Kolonien nach allen Richtungen durchsetzenden, einzelligen breiten Haare. Sie sind zweifellos von Bedeutung, doch über diese können nur Vermutungen geäussert werden.

Die Nostoc-Behälter machen den Eindruck von Lockfallen für die Alge und es wäre möglich, dass die erwähnten Haare durch bestimmte Ausscheidungen eine anziehende chemotaktische Wirkung auf die Alge ausüben. Auch die Möglichkeit ist nicht von der Hand zu weisen, dass die Haare vielleicht die von der Alge gebildeten N-Verbindungen aufzunehmen bestimmt sind und dem Moose zuführen.

Es dürfte kein Zufall sein, dass sich in den Blatthöhlen von Azolla neben der Anabaena ähnliche Haare vorfinden und schon Strasburger vermutet hier ernährungsphysiologische Beziehungen zwischen den Anabaenaschnüren und den zwischen diesen eindringenden Kolbenhaaren¹⁾.

---

1) Strasburger E., Über Azolla. Jena 1873, p. 39–40.

Derselben Meinung ist auch Oes[1], der mit grosser Wahrscheinlichkeit bewiesen hat, dass Azolla freien Stickstoff zu assimilieren vermag und vermutet, dass die Haare in den mit Anabaena erfüllten Blatthöhlen dabei eine Rolle spielen. „Die Eiweissreactionen der Keulenhaare, die die Azolla zwischen die endophytischen Anabaenen hineinsendet, berechtigen zu der Vermutung, dass Anabaena Azollae bei der Assimilation des freien Stickstoffs eine Rolle spiele. Es liesse sich, gestützt auf die mitgeteilten Beobachtungen und Versuche ein Symbioseverhältnis denken, wobei die Anabaena der Azolla N-Verbindungen liefern und dafür andere Stoffe z. B. Kohlehydrate empfangen würde. Die Keulenhaare scheinen hiebei als Brücke zu dienen."

Oes war es auffallenderweise nicht geglückt, die Anabaena Azollae in Reinkultur herauszuzüchten aber meinem Assistenten Herrn Dr. Limberger gelang nicht nur dies, sondern, wie ich mich selbst überzeugte, auch die Kultur von Azolla frei von Anabaena. Die von Anabaena befreite Azolla wächst sehr üppig und so möchte es, da es gelungen ist, Anabaena und Azolla unabhängig von einander zu ziehen auf den ersten Blick scheinen, als ob die beiden Pflanzen nicht viel Nutzen von einander hätten. Aber in Wirklichkeit könnte die Sache doch anders liegen, da wir ja auch die Flechtengonidien für sich rein kultivieren können und doch von einer Symbiose zwischen Alge und Pilz überzeugt sind.

Bekanntlich können auch insektenfressende Pflanzen ohne Insekten leben und doch wird heute kein Physiologe daran zweifeln, dass der Insektenfang den insektenfressenden Pflanzen sicher von Nutzen ist und einen wertrollen Nahrungszuschuss bedeutet.

4. Auch der Frage, was geschieht denn schliesslich mit der Nostockolonie im Thallus der Lebermoose, schenkte ich Aufmerksamkeit und kann darüber folgendes mitteilen. Der Thallus der Blasia verliert im Winter sein Chlorophyll, er vergilbt, und merkwürdigerweise vergilben auch die darin befindlichen Nostockolonien ; auch sie verlieren ihre grüne Farbe und werden gelblich. Ganz auffallend ist der Unterschied zwischen den grünen und vergilbten Nostoczellen.

Die grünen erscheinen mit graugrünem Zellinhalt prall gefüllt, zeigen das normale Aussehen und geben intensive Raspailsche Reaction.

---

[1] Oes A., Über die Assimilation des freien Stickstoffs durch Azolla. Zeitschr. f. Botanik. 1913, 5. Jg. p. 161.

Die vergilbten hingegen haben ihren Inhalt mehr oder weniger eingebüsst, sind grau oder bräunlich gelblich und geben auch nicht in Spuren die Raspailsche Reaction.

Die Unterschiede sind in die Augen springend. Es drängt sich dabei unwillkürlich die Ansicht auf, dass zu der Zeit, wenn die Blasia vergilbt, alle wertvollen, noch verwendbaren Stoffe zur Ausbildung der tiefgrünen, massenhaft erscheinden Brutkörper und der Früchte aus dem Thallusparenchym und den Nostockolonien entnommen und diese dabei grossenteils ausgesogen und verdaut werden.

Auch bei Behandlung mit Jodjodkalium zeigt sich im Verhalten der grünen und vergilbten Kolonien ein grosser Unterschied. Jene färben sich tiefbraun, diese gar nicht. Die Braunfärbung kann von Eiweiss oder Glykogen oder von beiden herrühren.

ERKLÄRUNG DER TAFEL

Blasia pusilla.

Fig. 1. Thallus mit den flaschenförmigen Brutbehältern und den Nostockolonien n. Vergr. 4.
Fig. 2. Dauer-Brutkörper mit anhängendem Stiel s. Vergr. 312.
Fig. 3. „Sternschuppe" mit 2 Nostocherden in den Blattohren. Vergr. 123.
Fig. 4. Eine Nostockolonie im Blattohr mit den Haaren h. Vergr. 500.
Fig. 5. Ein Teil einer solcher Nostockolonie mit den Haaren h, etwas gequetscht. Vergr. 500.
Fig. 6. Eine Reinkultur der Blasia-Nostoc in einer Proberöhre auf Agar. 5 Monate alt. Natürl. Grösse.
Fig. 7. 3 epiphytische Nostockolonien von Sternschuppen Vergr. 625.
Fig. 8. Thallusstück mit Nostockolonien und massenhafter Ablagerung von CO_3Ca in Form von dunklen Streifen s. Vergr. 16.
Fig. 9. Stücke des CO_3Ca. Vergr. 312.

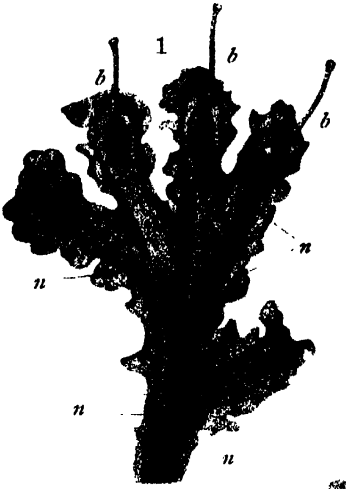
ERKLÄRUNG DER TAFEL.

Cavicularia densa.

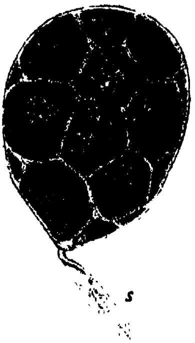
Fig. 1. Thallusstück mit Nostoccolonien n, in Längsreihen rechts und links von der Mitte. Vgr. 4.
Fig. 2. Thallusstück mit Nostoccolonien n und Brutkörperbehältern bb, Vergr. 4.
Fig. 3. Nicht ruhender Brutkörper, bereits inficiert mit 2 Nostoccolonien nn, Vergr. 128.
Fig. 4. Ruhender Brutkörper. ii der Sitz der Initialen; s Stiel. Vergr. 123.
Fig. 5. Zerdrückte Nostoccolonie mit den Kolbenhaaren h. Vergr. 500.
Fig. 6. Reinkultur von Nostoc auf Agar. c Kolonien. Natürl. Grösse.
Fig. 7. Querschnitt durch den mittleren Teil des Thallus mit 4 Parenchymbündeln p, die CO_3Ca führen. Unten Rhizoiden r. Vergr. 50.
Fig. 8. Stücke von CO_3Ca aus den Parenchymbündeln. Vergr. 812.

HANS MOLISCH.

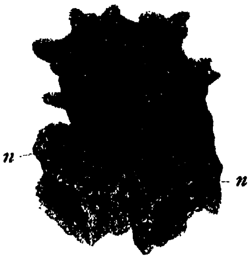
1



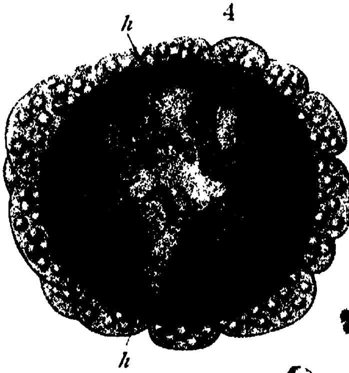
2



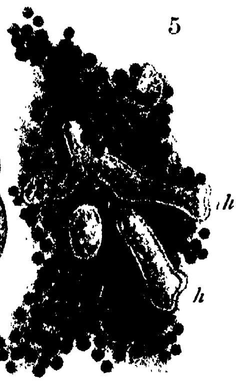
3



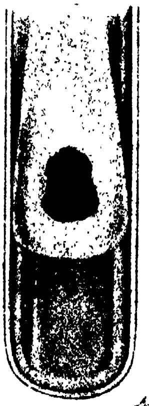
4



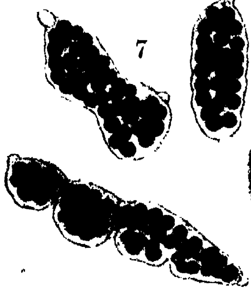
5

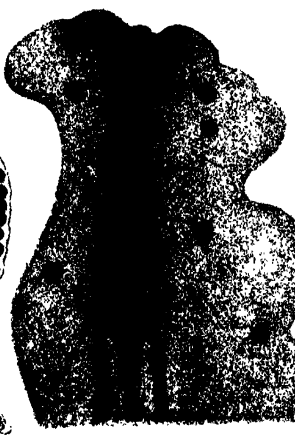


6

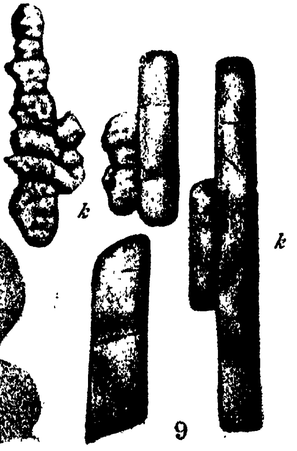


7





8



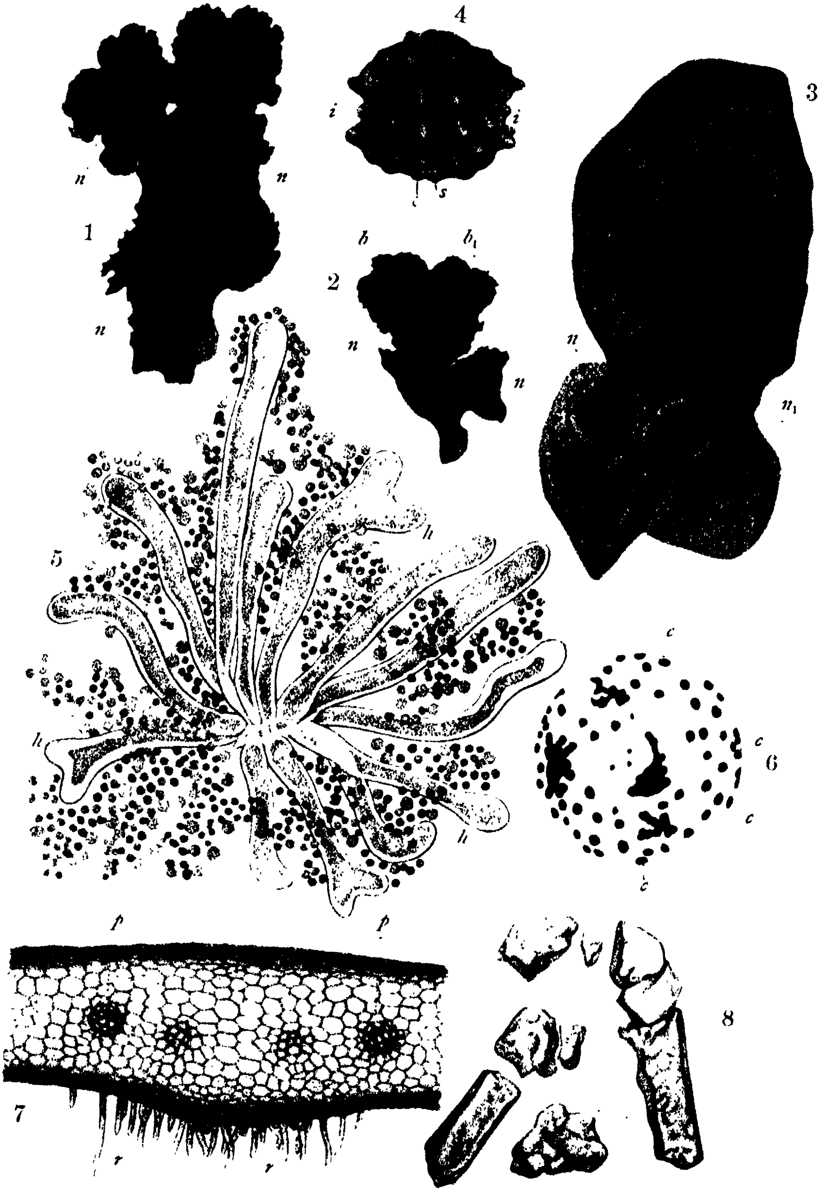
9

(B. Sakuma del.)

SYMBIOSE DER LEBERMOOSE

HANS MOLISCH.

TAFEL II.



(B. Sakuma del.)

MITOSEN BEI SARGASSUM.

VON

MASATO TAHARA UND NAOMASA SHIMOTOMAI.

Durch die Mitteilung von Nienburg[1] veranlasst, teilte einer von uns im vorigen Jahre einen Befund über die Embryobildung von Sargassum mit.[2] Die Eizelle dieser Alge besitzt nämlich im Beginn ihrer Entwicklung zwei grosse Kerne, einen im Zentrum, den anderen im unteren Pole. Nienburg hat seinerzeit den unteren für einen in Degeneration begriffenen gehalten. Dies ist aber nicht der Fall. Bei der weiteren Entwicklung teilen sich diese zwei Kerne in gleicher Weise, sowohl der obere wie der untere, ganz normal im mitotischer Weise. Also kamen wir damals zum Schlusse, dass das Ei von Sargassum merkwürdigerweise zwei Eikerne in sich beherbergt. Da es aber sehr unwahrscheinlich ist, dass diese beiden Kerne oder der eine von ihnen durch die Samenkerne befruchtet werden, sind uns Zweifel aufgestiegen, ob überhaupt eine Befruchtung bei dieser Alge vorkommt. Um diesen Punkt aufzuklären, begaben wir uns wieder in diesem Frühling nach Misaki zur Biologischen Station der kaiserlichen Tokio Universität. Wie schon bekannt, schreitet die Entwicklung des Oogoniums dieser Alge bestimmt im allgemeinen periodisch und simultan fort. Darum kann man das betreffende Material nur zu gewissen Stunden an gewissen Tagen gewinnen. Zuerst hatten wir geglaubt, dass die von uns gewünschten Stadien, d. i. die meiotischen Phasen des Oogoniumkerns nur in der Nacht zu kommen pflegen. Dies ist aber nicht richtig. Weitere Erfahrungen haben uns gelehrt, dass diese Stadien manchmal auch am Tage auftreten. In Misaki wuchern verschiedene Arten von Sargassum. Wie bei der vorigen Arbeit, haben wir auch diesmal hauptsächlich nur Sargassum enerve benutzt. Da die Einzelheiten über die Teilungsvorgänge des Oogoniumnukleus in der nächsten Mitteilung genauer berichtet werden, möchten wir hier nur die Tatsache betonen, dass die ersten zwei aufeinander folgenden Kernteilungen im Oogonium in natura ganz und gar meiotisch sind. Fig. 1 a und b zeigen die Pol- und Seitenansicht der ersten, also heterotypischen Kernteilung im Oogonium. Die eine von diesen Figuren, die Polansicht, wurde etwas stärker vergrössert gezeichnet als die andere. In dieser Teilung kommen Zentrosomen, je eins an jedem Pole, immer sehr deutlich vor. Die Chromosomen sind viel dicker als die der somatischen Kernteilung. In der Polansicht lassen sich ohne Schwierigkeit 32 Chromosomen zählen. Die Haploidchromosomenzahl von Fucus vesciculosus beträgt nach Yamanouchi[1] auch 32. Man würde also vielleicht berechtigt sein, anzunehmen, dass die gesamten Arten von Fucaceae dieselbe Chromosomenzahl 32 zeigen.

---

[1] Nienburg, W., Die Oogonentwicklung bei Cystosira und Sargassum. (Flora, Neue Folge, Bd. 1910, S. 170.)

[2] Tahara, M., Zur Kenntnis der Keimentwicklung bei Sargassum. (Science Reports of the Tohoku Imperial University Vol 1, No. 1. 1924. p. 91.)

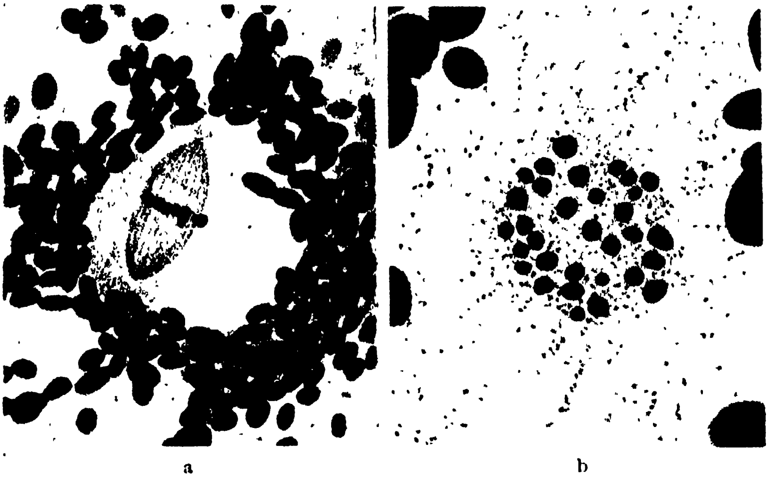


Fig. 1. a. Seitenansicht der heterotypischen Kernteilung im Oogonium (×1500).
b. Die Polansicht derselben Teilung (×3200).

Die somatischen Kernteilungen von Sargassum können bei der Keimentwicklung leicht beobachtet werden, aber es ist ziemlich schwer, eine gute Polansicht zu finden, welche die Chromosomenzahl von dieser Alge klar zu bestimmen erlaubt. Also leider können wir die diploide Chromosomenzahl von S. enerve hier nicht exakt angeben. Es kann jedoch nicht bezweifelt werden, dass diese etwa 60 beträgt.

---

[1] Yamanouchi, S., Mitosis in Fucus. (Bot. Gaz. Vol. XLVII, 1909. p. 173.)

Das oben erwähnte Ergebnis über die meiotischen Teilungen im Oogonium hat die Vermutung betreffs der Parthenogenese dieser Alge

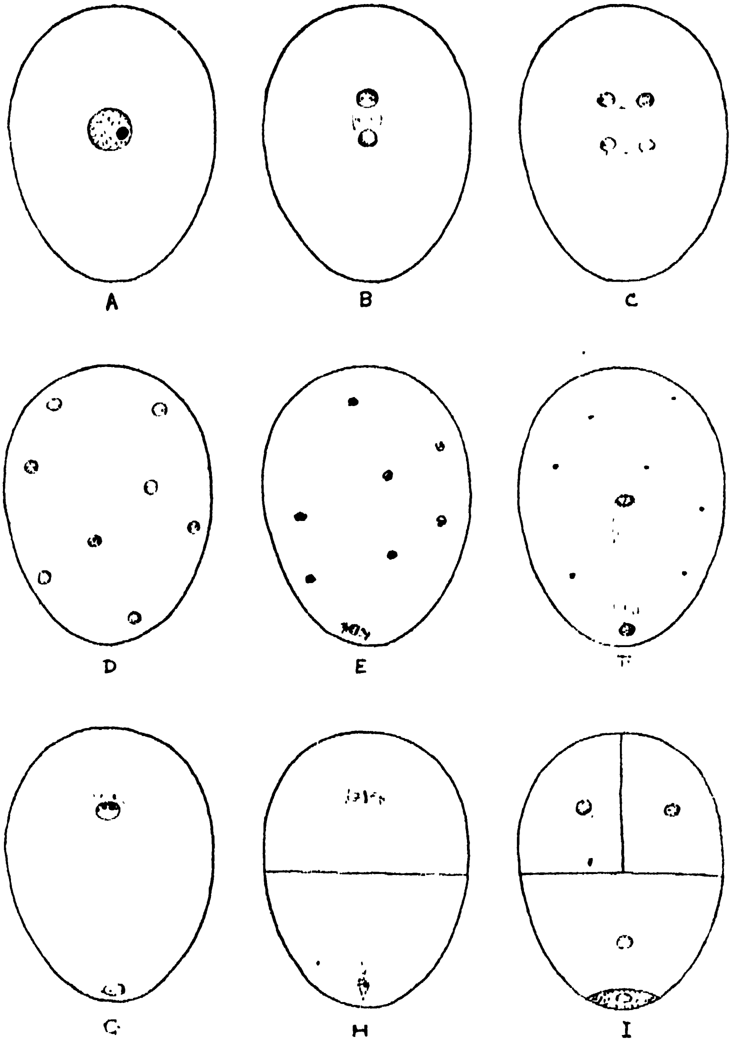


Fig. 2. Schematische Darstellung der Ei- und Keimentwicklung von Sargassum. A-C. Meiotische Teilungen im Oogonium. D. Achtkernigs Stadium. E. F, Erste Teilung in der Keimentwicklung. II. Prophase der zweiten Teilung. H. Metaphase derselben Teilung. I. Vierzelliges Stadiums des Keimes.

vernichtet. Es erschien nun sehr wünschenswert, die ersten Stadien der Keimentwicklung mit höchster Sorgfalt noch einmal zu untersuchen. Neuerdings wurde eine Biologische Station der hiesigen Universität in Asamushi, nahe bei Aomori, eingerichtet. Diesen Sommer hatten wir das Glück, dort genügendes Material für weitere Untersuchungen zu gewinnen. Die Meeresvegetation in Asamushi ist etwas anders als die in Misaki. Diesmal fixierten wir hauptsächlich Sargassum tortile. Eine ganz unerwartete Erscheinung haben wir bei dieser Alge gefunden. Durch die drei aufeinander folgenden Teilungen entstehen zuerst acht Kerne in jedem Oogonium. Bisher haben wir bloss angenommen, dass von diesen acht Kernen sechs zu Grunde gehen und zwei sich definitiv zu Eikernen ausbilden. Das war aber eine grosse Täuschung. Nicht sechs, sondern sieben degenerieren, und im Momente dieser Degeneration führt der einzig intakt bleibende Kern eine nochmalige mitotische Teilung aus. Diese Teilung verläuft in etwas eigentümlicher Weise. In der Anaphase trennen sich die zwei Töchterchromosomklümpchen sehr rasch voneinander. Also schon in der Telophase liegen die zwei neu entstandenen Töchterkerne in grosser Entfernung. Es ist bemerkenswert, dass diese Teilung immer in der Peripherie der Eizelle, nicht in ihrem Zentrum ausgeführt wird und der eine von diesen zwei Kernen immer in der Peripherie bleibt und der andere sich nach innen zu bewegen pflegt.

Leider können wir hier nicht sagen, wo und wie die Befruchtung bei dieser Alge stattfindet. Aber unserer Meinung nach dürften die in dieser Weise entstehenden zwei Kerne schon von Anfang an mit diploiden Chromosomen begabt sein und können nicht mehr Eikerne genannt werden. Hoffentlich werden wir in der nächsten Mitteilung alles klar zu machen imstande sein. Die etwas schematisch gezeichneten Textfig. 2, A-I werden die aufeinander folgenden Stadien der Ei- und Keimentwicklung dieser Alge verständlich machen.

Zum Schlusse möchten wir Herrn Professor Dr. Naohide Yatsu, Direktor der biologischen Station zu Misaki und Herrn Professor Dr. Shinkishi Hatai, Direktor der biologischen Station zu Asamushi unsern verbindlichsten Dank für ihre freundliche Unterstützung aussprechen.

ON THE AQUATIC OLIGOCHÆTE, TUBIFEX HATTAI, N. SP.

By

EKITARO NOMURA.

The aquatic oligochæte, which has not yet been described, is found in gutters and ditches of Tokyo and Sapporo. It shows the main characters of Tubifex and I propose the name Tubifex hattai. The new name is chosen to express my sincere regard for Prof. S. Hatta, Professor of Zoology, Imperial University of Hokkaido.

Before taking up the subject of this paper I wish to express my heartfelt thanks to Prof. S. Goto for his helpful advice, and to Prof. S. Hatai for kindly giving me access to his library.

I. DESCRIPTION.

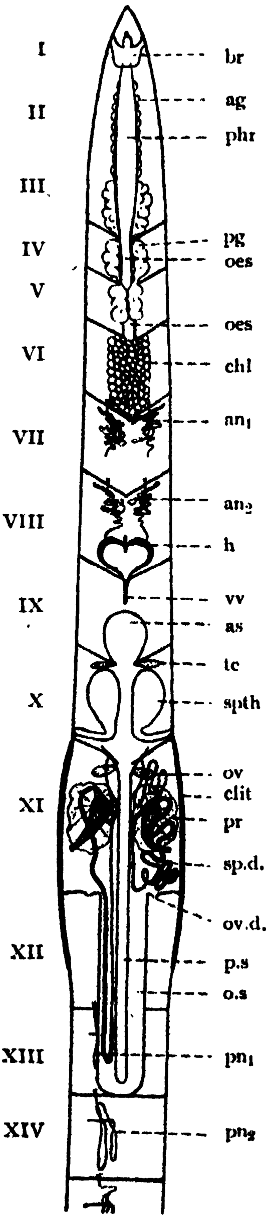
Colour of the body pinkish yellow, shaded posteriorly with light gray. Number of segments 85–110. Body length above 10 cm. in fully mature and well narcotized, July specimen. Clitellum forming a nearly complete ring, occupying Segment XI and anterior two-thirds of Segment XII. Clitellar portion the widest, easily distinguished from the succeeding portion, and measuring about 1 mm. or more. Preclitellar portion, diminishing in width somewhat rapidly and ending with a conically pointed prostomium. Postclitellar portion gradually diminishing in width posteriorly to the last segment.

Brain squarish, emarginate posteriorly.

Setæ sigmoid and capillary, preclitellar dorsal sigmoid bifurcate and uni- to tetra-dentate; postclitellar and ventral sigmoid invariably bifurcate; both branches of bifurcate sigmoid of about equal size. Each dorsal seta-bundle consists mostly of four sigmoid and three capillary setæ in the preclitellar segments, fewer posteriorly. Each ventral seta-bundle consists mostly of four sigmoid setæ in the preclitellar segments, also fewer posteriorly.

Septal sacs attached to the posterior faces of Septa V/VI—?XIV/XV, on the ventral side of the alimentary canal; anterior ones better developed.

Fig. 1.



Diagrammatic representation of the principal organs. Segments I—VIII are seen from the ventral side and Segments IX—XIV from the dorsal side. One of the sperm-ducts shown on the left side of this diagram shows that it is extended into the posterior ovisac and that the ciliated and non-ciliated portions are of nearly equal length. I—XIV—numbers of segments, ag—anterior pharyngeal gland, an_{1-2}—first and second anterior nephridia, as—anterior sperm-sac, br—brain, chl—chloragogues, clit—clitellum, h—heart, oes—œsophagus, o. s—ovisac, ov—ovary, ov. d—oviduct, pg—posterior pharyngeal gland, phr—pharynx, pn_{1-2}—posterior nephridia, pr—prostate gland, p. s—posterior sperm-sac. sp. d - sperm-duct, spth—spermatheca, te—testis, vv—ventral vessel.

Pharynx in Segment II. Oesophagus in Segments III—V. Pharyngeal glands well developed in Segments III—V. Intestine and chloragogues commence in Segment VI.

Anterior nephridia in Segments VII and VIII, paired. Posterior nephridia commence in Segment XIII, those of the right side being invariably absent.

Intestinal hearts in Segment VIII. Dorsal and supraintestinal vessels separately present anterior to Segment IX; dorso-supraintestinal vessel from Segment IX to the last. Subintestinal vessel present anterior to Segment XI; ventral vessel present, discontinues in Segments VIII and IX; segmentary subintestinal vessels in segments posterior to XII.

Testes in Segment X. Ovaries in Segment XI. Sperm-sacs unpaired; anterior one in Segment IX; posterior one in Segments XI—XIII. Ovisac in Segments XII—XIII. Sperm-ducts very long, about four times as long as the longest segment of the body, viz. XI, or longer. Each vas deferens consists of a proximal, narrow, cilliated, and a distal, wide, non-ciliated portion; both of about equal length. Atrium about half as long as Segment XI, its distal portion continued to the penis without a distinct, intervening duct portion. Prostate gland large, irregular in shape. Penis short, conical, without chitinous sheath. Spermathecæ in Segment X, paired, saccular in shape, opening laterally midway between the dorsal and ventral seta-bundles on either side of the body. Spermatophores present.

Habitat—Tokyo, Sendai, Morioka, Sapporo.

II. ANATOMY AND HISTOLOGY.

The materials were narcotized with chloreton and fixed with acetic sublimate.

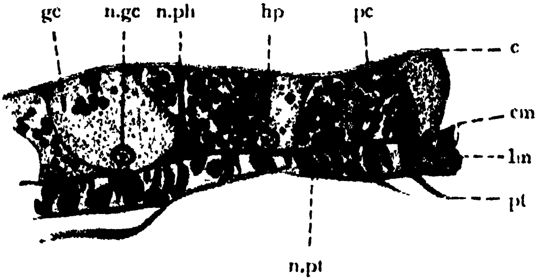
1. BODY WALL, SEPTA, SEPTAL SACS AND INTERSEGMENTAL ORGANS.

1) The body wall is generally thin except in the anterior portion of the body. "Die ersten 5 Segmente," writes Vejdovsky (1884) for his Tubifex rivulorum, "in zwei ungleiche Ringel geteilt, von welchen das hintere, breitere, die Borsten trägt." Dixon (1915) denies, however, the presence of annuli in the same species and states that "The worm never exhibits any 'secondary' annulation of the segments, such as occurs so generally in Limnodrilus." In living specimens of Tubifex hattai, when the animal is retracting its body, annuli are formed on some of the anterior segments. Segment I is always single. Segment II consists of a shorter anterior and a longer posterior annulus. Segments III and IV consist of three annuli, increasing in length posteriorly. The longest hindermost annuls in these segments always bears the seta-bundles.

The outermost cuticular layer is very thin, measuring about 0.5 μ, and structureless. The pore canals or the openings of the hypodermal gland cells measure 1–1.5 μ, in diameter.

The extraclitellar hypodermis measures in the preclitellar portion 3.5–5 μ, and in the postclitellar portion less than 3 μ. The ordinary hypodermal cells contain a compact granular cytoplasm and the cell-boundaries are observed only at both ends of the worm. The nuclei mostly are compact and discoidal, being scattered in the middle of the hypodermal layer, but at setal regions and at both ends of the body they are rather roundish and more or less vesicular in appearance. The hypodermal gland cells are comparatively abundant in the anterior segments, but fewer in the posterior. There is no regularity in their arrangement. Each gland cell contains a feebly staining and finely granulated cytoplasm and a discoidal nucleus at its base.

Fig. 2.



Cross section through the clitellum of a mature specimen. c—cuticle. cm—circular muscle, gc—clitellar gland cell, hp—supporting cell, lm—longitudinal muscle, n. hp—nucleus of supporting cell, n. gc—nucleus of clitellar gland cell, n. pt—nucleus of peritoneal cell, pc—pore canal, pt—peritoneum.

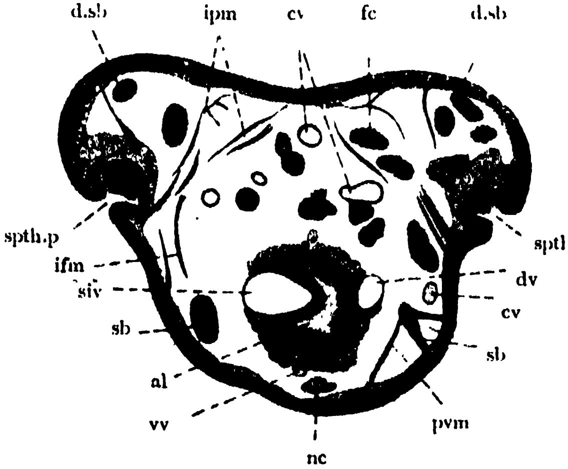
The clitellum is thinner on the ventral side than on the dorsal and shows the ordinary structure of the hypodermis at the setal regions and the genital pores. Beside these a slight depression of the ordinary structure is seen on the ventral median line of Segment XI. It is a long elliptical depression extending from near the anterior septum to about the middle point between the male pores. The clitellar hypodermis is very thick, measuring 15–20 μ, and is highly glandular; it consists of large clitellar gland cells and strongly compressed supporting cells. The clitellar gland cells vary in form according to the stages of their activity. Each young gland cell is cubical or low prismatic and contains a feebly staining cytoplasm and small granules stained deeply with eosin. In a more advanced stage, the small granules aggregate and unite into globules of different sizes but always less than 3 μ in diameter. These globules take hæmatoxylin in hæmatoxylin-eosin stain; congo-red in hæmatoxylin-congo-red stain, the cytoplasm rather taking hæmatoxylin; and carmine strongly in picro-carmine.

So these globules are easily distinguished from yolk granules which always take only plasma-stains. The fully developed gland cells are mostly spherical and again return to their initial state. In Tubifex rivulorum these cells at their maximum development may be as much as 40–45 μ long, and 11–14 μ across (Dixon, 1915). The nucleus of each clitellar gland cell is greater than that of the extraclitellar, spherical or ellipsoidal, nucleolated and situated at the proximal half of a cell. The nucleus of each supporting cell is strongly compressed, non-nucleolated and situated at the middle of the cell.

The muscle layers are not so well developed even in the anterior segments. Especially in the posterior segments the longitudinal muscle fibers can hardly be detected in cross sections.

The peritoneum is well developed in the anteriormost segments and sometimes twice as thick as the longitudinal muscle layer and has a cellular structure, but in most portions of the body it is of about the

Fig. 3.



Cross section through spermathecal pores. al--alimentary canal. cv--coelomic commissural vessel, d. sb—dorsal seta-bundle, dv—dorso-supraintestinal vessel, fc—floating sperm-cells, ifm--interfollicular muscle, ipm--interparietal muscle, nc—ventral nerve cord, pvm--parietovaginal muscle, spth. p—spermathecal pore, sb--ventral seta-bundle, siv—subintestinal vessel, vv—ventral vessel.

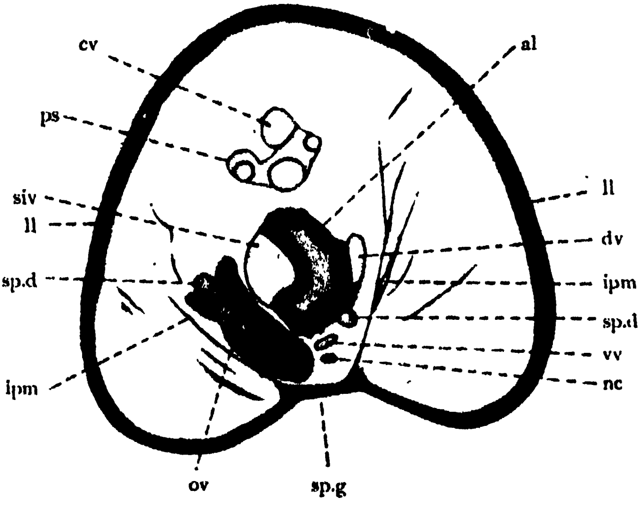
same thickness as the longitudinal muscle layer and shows a fibrous appearance. The inner surface of the peritoneum is generally smooth; the nuclei mostly are fusiform or ellipsoidal, nucleolated or non-nucleolated and always situated near the inner surface.

The lateral lines can not be detected in the posterior segments, but in the anterior they are quite distinct from the peritoneum. In Tubifex rivulorum Dixon (1915) could not find absolutely surely the lateral lines, but Vejdovsky (1884) gives a fine figure of this structure.

The outline of the body as seen in cross sections of Segments X and XI is notable for its special shape in connection with the muscle fibers.

In a cross section through the spermathecal pores or the seta-bundles of Segment X the outline of the body shows a wider dorsal and a narrower ventral half (Fig. 3). This is caused by the interparietal muscle fibers which run out obliquely from the dorsal median wall and are attached to the lateral wall near the spermathecal pore or the lateral line on either side of the body.

Fig. 4.

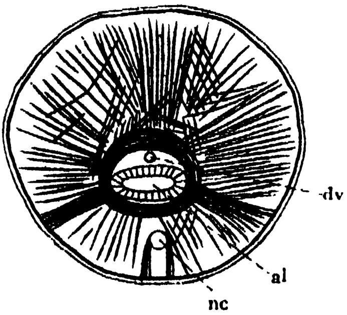


Cross section through the anterior portion of Segment XI. al—alimentary canal, cv—cœlomic commissural vessel, dv—dorso-supraintestinal vessel, ipm—interparietal muscle, ll—lateral line, nc—ventral nerve cord, ov—ovary, ps—posterior sperm-sac, siv—subintestinal vessel, sp. d—ciliated portion of vas deferens, sp. g—clitellar depression, vv—ventral vessel.

Segment XI shows just the reverse of Segment X and is narrower in its dorsal than in its ventral half, and somewhat triangular in shape as a whole (Fig. 4). This is due to the interparietal muscle fibers which connect the lateral to the ventral median wall. These fibers are well developed in connection with the clitellar depression, where they are attached not only to the ventral wall but also to the dorsal giving rise to a deep ventral indentation. In the male pore region the inlet vanishes and the ventral wall becomes flat, the male pores opening directly on the ventral surface. A similar relation can be seen in connection with the female pores.

2) The septa begin at the intersegment III/IV. In the preclitellar segments they are always funnel-shaped, with the smaller end directed posteriorly, while in the postclitellar segments they are all set transversely to the intestine. The septa are generally very thin and contain a feebly developed septal musculature. The musculature of the preclitellar septa can be distinguished into four groups according to their functions; circular fibers surrounding the alimentary canal; semicircular ones binding the ventral nerve cord to the ventral body wall; radial ones connecting the intestinal circular muscle to the body wall; and lateral ones connecting the intestine to the body wall (Fig. 5). In the postclitellar septa the circular and the lateral muscle fibers are not developed and more posteriorly only the radial fibers are seen starting directly from the intestinal wall.

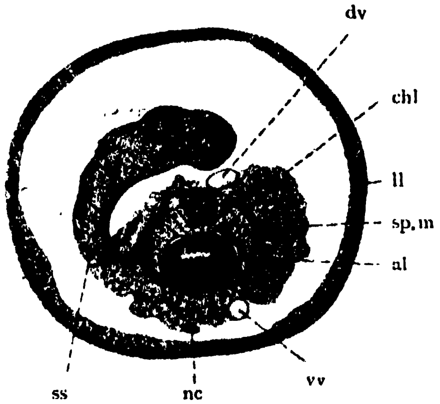
Fig. 5.



Septal muscles from an anterior segment. Reconstructed from sections. al—alimentary canal, dv—dorsal vessel, nc—ventral nerve cord.

3) The septal sacs are attached in pairs to the posterior faces of Septa V/VI—X/XI, on the ventral side of the intestine. They are developed in different degrees according to the degree of sexual maturation, but generally those of Septa VI/VII and VII/VIII are the largest, and those of Septum V/VI next in size, while those of Septa VIII/IX—X/XI are smaller. In the Sapporo-specimens additional pairs

Fig. 6.



Cross section through Segment VII just behind Septum VI/VII, to show hypertrophied septal sac. al—alimentary canal, chl--chloragogues, dv- dorsal vessel, ll--lateral line, nc—ventral nerve cord, sp. m—septal muscle fibers, ss—septal sac, vv—ventral vessel, which is united with the subintestinal vessel at the middle of the segment.

of septal sacs are found posteriorly to Septum XIV/XV and the ones behind Septum IX/X are also well developed. The septal sacs ordinarily are developed equally on both sides of the body, but sometimes one of a pair may atrophy and when this is the case the remaining one is hypertrophied and reaches even to the dorsal side of the intestine (Fig. 6).

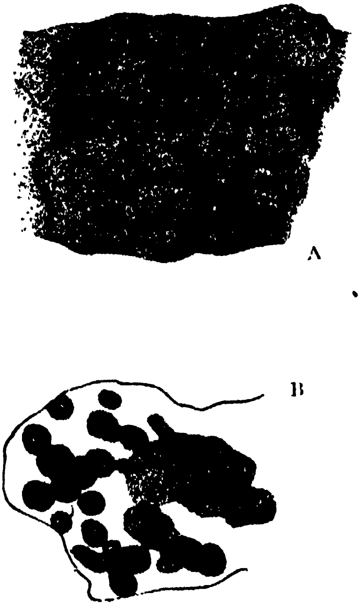
Each septal sac consists of a thin structureless membrane and numerous cells contained in it (Fig. 7). The bottom cells are mostly small and spherical and contain a compact cytoplasm of embryonic appearance having a great affinity for hæmatoxylin in hæmatoxylin-eosin stain and congo-red in hæmatoxylin-congo-red stain. These cells are very similar in appearance to those of Limnodrilus gotoi, which are taken by Stephenson (1922) to be chromophil cells. But they are nevei chromophil cells, so far as he defines them to be stained deeply only with nuclear dyes. The cells at the entrance are irregularly shaped, aggregated compactly and look like a glandular tissue. The cytoplasm always takes plasma-stains. The nuclei of both forms of cells are spherical and nucleolated.

4) The intersegmental organs are fairly large sized cell masses found on the anterior face of each septum behind XII/XIII, directly dorsal to the intestine. In living specimens they can be easily distinguished under a low power from the chloragogues by their compactness and transparency. They are pear-shaped, attached to the septum by the narrow end and are also observable in total preparations stained with borax-carmine. In sections, each one is seen to receive a few

muscle fibers from the septal musculature but is not connected with any other organ. Each constituent cell is generally rather small, roundish and contains a compact nucleus, but sometimes it attains to an extraordinary size, with two or more (sometimes 5 or 6) nuclei, and may be pear-shaped and contain a comparatively large vacuole in the distal half. The cytoplasma always take up plasma-stains.

The above refers to the ordinary intersegmental organs, but some notable variations are often met with. In one case the organ was formed by an anterior bulging of the septum and covered by peculiar cells, and in another case the sac was directed posteriorly and looked just like a septal sac with its contained cells. From these facts I conclude that the organ is primarily nothing but the outgrowth of a septum. Moreover since its position is the same as that of the genital sacs and, since when the latter are well developed the intersegmental organ attached to Septum XII/XIII disappears, the organs in question are perhaps homologous with the genital sacs.

Fig. 7.



Sections to show cells contained in a septal sac. In A are shown cells at the entrance and in B bottom cells.

The cœlomic commissural vessels enter the intersegmental organs as soon as they leave the dorso-supraintestinal vessel.

"The cœlom," writes Dixon (1915) of her Tubifex rivulorum, "is always filled with a colourless cœlomic or perivisceral fluid, by which all the organs of the body are constantly bathed. Suspended in the fluid are a number of colourless corpuscle which may be described as cœlomic or perivisceral corpuscles. The number of these corpuscles in different individuals varies enormously, but two kinds are usually recognizable—amœboid and spherical." In Tubifex hattai these corpuscles are very rarely observed.

2. SETÆ AND SETA-BUNDLES.

As to the setæ of Tubifex rivulorum they are described in different ways by different authors.

Dorsal setæ—

"Die Rückenborsten der vorderen 15–16 Segmente in 3 Zähnchen getheilt oder undeutlich kammförmig, zwischen welchen noch Haarborsten sich finden. Vom 16. Segmente an bis zum hinterem Körperende sind nur gegabelte Borsten mit gleich langen Zähnchen.——— Tubifex rivulorum ist in dem äusseren Habitus des Körpers nach seinem Wohnorte äusserst veränderlich. Die im sandigen Grunde der reinen, fliessenden Bäche lebenden Würmer sind lebhaft roth, dünn, mit eigenthümlich gekrümmten undeutlich kammförmigen Borsten" (Vejdovsky, 1884).

"Integument furnished with only two kinds of spines, viz. hair-spines and forked spines. Fan-shaped or pseudo-comb-like spines never present" (Eisen, 1885).

"Dorsale Bündel am 2–15. Segm. mit gleichzinkig gegabelten Hakenborsten, die ein Mittelzähnchen oder einen undeutlichen Kamm zwischen den beiden Zinken tragen, sowie mit Haarborsten; am Mittelkörper und Hinterkörper nur mit einfachen, gleich-zinkig gegabelten Hakenborsten" (Michaelsen, 1900).

"Three kinds of setæ are present, viz. capilliform, uncinate, and pectinate. The distribution of the capilliform setæ is limited, for they are confined to the dorsal bundles, and do not usually extend further back than the 30th segment. The uncinate setæ are common to both dorsal and ventral bundles throughout the body, while the pectinate form is limited to the dorsal bundles of the anterior segments, and is absent from some of these.———The uncinate setæ in the dorsal bundles have the two prongs equal in size and of the same shape, that is, somewhat narrow throughout and tapering gradually to a very fine point at the dorsal end.———The pectinate setæ resemble the uncinate of the dorsal bundles in general form. The only difference between them is the presence, in the pectinate form, of subsidiary prongs between the two main divisions of the seta.———Their number (subsidiary prongs) varies from 1 to 4, the latter number occurring most rarely. I have not yet been able to identify the webbed setæ described by Lankester" (Dixon, 1915).

From the above descriptions only one common point is made sure; that is that the dorsal bifurcate setæ of Tubifex rivulorum have branches of equal size.

Ventral setæ—

"Die Bauchborsten durchaus von gleicher Form, gegabelt, das obere Zähnchen länger als das untere" (Vejdovsky, 1884).

"Ventrale Bündel mit gabel-spitzigen Hakenborsten, deren obere Zinke stets länger als die untere ist. ....Geschlechtsborsten fehlen" (Michaelsen, 1900).

"In the ventral bundles, where the uncinate setæ only are present, the two prongs are of slightly different size and shape. The dorsal one is somewhat slender, and comes to a sharp point at the tip. The ventral one is shorter than the dorsal, and of a blunter nature, its apex being somewhat more rounded" (Dixon, 1915).

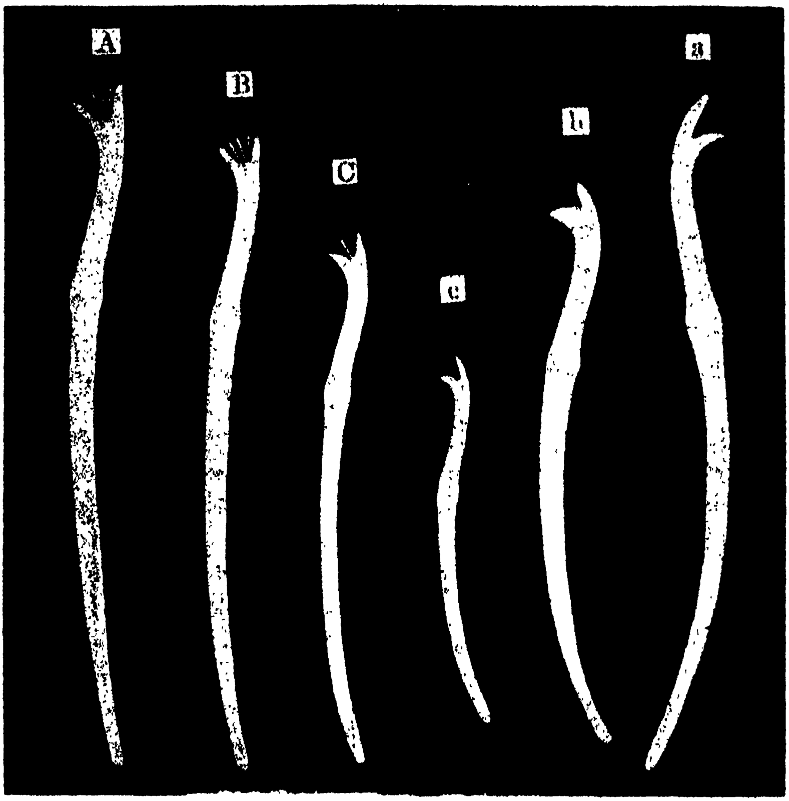
These descriptions coincide on the point that each ventral seta of Tubifex rivulorum has a longer, upper and a shorter, lower branch.

Three kinds of setæ are found in Tubifex hattai.

1) Bifurcate sigmoid setæ. This form is most widely distributed on the body. The dorsal setæ in most segments behind the clitellum and all ventral setæ belong to this category. The upper branch of each seta is generally longer and more slender than the lower, but some of the ventral setæ on the middle segments sometimes have the upper branch shorter than the lower. The nodule is smooth and lies in most setæ at two-thirds setal length from the proximal end. The largest setæ measure almost 145 μ and are found on Segments III—VIII, but on the posterior segments those of only 80 μ are common.

2) Dentate sigmoid setæ. This form is found only in the dorsal seta-bundles of the anterior segments. It appears somewhat thinner and seems to have less sigmoidal flexure than the first. The nodule is smooth and lies at about two-thirds setal length from the proximal end. The portion between the nodule and the tip is seemingly stronger than in the bifurcate form. The tip bears 1–4 middle teeth between the principal branches. The latter are nearly straight and appear of equal size even under a high power. Each middle tooth is conical

Fig 8.



Setæ. A B C from dorsal seta-bundles and a b c from ventral. A—the largest from Segment V, B—the largest from Segment III, C—the largest from Segment X, a—the largest from Segment III, b—the largest from Segment XV, c—a small complete seta from Segment LXX.

or rather bat-like, and is attached by its narrow end to the bottom of the furcation. The number of middle teeth vary even in the same seta-bundle and generally the oldest seta, which is found near the dorsal median line of the body, has more middle teeth than the younger. The dorsal setæ of Segments II—VIII are mostly tri- or bidentate, while those of Segments IX—XII are unidentate or simply bifurcate. The tetradentate ones are found in most cases in Segments V and VI only. The furcation of a unidentate seta is closely similar to that of the bifurcate. The dorsal sigmoid setæ are always shorter than the ventral of the same segment.

3) Capillary setæ. These are confined to the dorsal bundles and to the front half of the body. Each one is thickest at its proximal end and gradually narrows to the tip. It is always thinner than the sigmoid of the same bundle, and it may measure up to 0.8 mm. On Segments III—VIII these setæ are noticeably longer and can be seen with the naked eye when they move about with fragments of algæ.

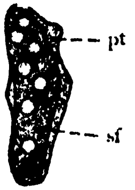
The seta-bundles begin in Segment II. In the preclitellar segments except X they are situated rather in the middle of the segment, but further back they lie in the posterior half; anterior to about Segment XV three-fifths segment length from the anterior septum, and posterior to the same two-thirds.

The four seta-bundles of a preclitellar segment lie almost at the corners of a squarish cross section, but in the segments behind the clitellum generally the dorsal bundles lie more apart than the ventral ones and in the posteriormost segments they regain the former position.

The number of setæ in a ventral seta-bundle anterior to Segment XIII, except XI, is generally 4, and to about Segment XXV 3; fewer posteriorly. The dorsal seta-bundles of the anterior segments, except XI, always contain capillary setæ. The number of sigmoid setæ is in Segment II 3, in Segments III—X generally 4 sometimes 5, in Segments XII—?XXX generally 3, and more posteriorly 2–1. The seta-bundles of Segment XI, both dorsal and ventral, consist of only 2 ordinary bifurcate setæ. Most seta-bundles usually contain 1 or 2 young setæ. The number of capillary setæ is in segments anterior to the clitellum mostly 3, rarely 2 or 4; fewer posteriorly.

The setigerous organ is of the form commonly seen in Tubificidæ, with three sets of muscles, viz. interfollicular, longitudinal and transverse parietovaginal muscles. When a seta-bundle contains capillary setæ, they are always situated posterior to the sigmoid setæ, and alternating with them (Fig. 9).

Fig. 9.



Cross section of preclitellar, dorsal setigerous organ. Anterior four are sigmoid and thicker, posterior three capillary and thinner. pt—peritoneum, sf—setigerous follicle.

3. ALIMENTARY CANAL.

The mouth lies ventrally at the beginning of Segment I. The buccal cavity reaches to the beginning of Segment II and the lumen presents in cross section a ∧-shape. The five layers of the body wall, except the hypodermis, are more feebly developed in the buccal cavity.

The pharynx is confined to Segment II. It is triangular in cross section, with triradiate lumen. The pharyngeal wall consists apparently of two layers: the inner endoderm of ciliated columnar cells and the outer thin peritoneal connective tissue, which contains a fine vascular network and feebly developed muscle fibers. At the beginning of the pharynx the endoderm is as thick as the hypodermis of the buccal cavity, but a little further backwards it is more or less suddenly thickened and becomes about three times as thick or thicker. The cytoplasm of the pharyngeal endoderm shows numerous fine strands running perpendicularly to the wall. The nuclei are ellipsoid or fusiform, nucleolated and are situated at the proximal half of the cell. The cilia are stouter and shorter in the dorsal median inlet than in the ventral. Many parietovisceral muscle fibers run out radially from the dorsal wall of the pharynx to the dorsal wall of the body; they are especially numerous at the beginning.

The pharyngeal glands are well developed and are distinguished into two kinds, the anterior and the posterior.

The anterior pharyngeal glands are irregularly arranged cell-masses attached to the dorsal surface of the pharynx. Each mass consists of many or sometimes a few gland cells. Each gland cell is pear-shaped and contains a somewhat granulated cytoplasm, which always takes up congo-red in hæmatoxylin-congo-red stain, and a large nucleolated nucleus in the distal half; its proximal end is prolonged into a duct, which either unites with others coming from neighboring cells or opens directly into the pharyngeal cavity through the interspaces of the endodermal cells.

The posterior pharyngeal glands are paired, notably large cell-masses enclosed in a thin peritoneal membrane. They begin at the posterior end of the pharynx and reach to the posterior region of Segment V. In Segment III they lie on the dorsal side of the œsophagus suspended by the parietovisceral muscle fibers. In Segment IV each gland lies on the lateral side of the œsophagus, with a dorso-ventral extension, but as it approaches the posterior septum it gradually shifts towards the ventral median line, and in Segment V it is mainly situated on the ventral side of the œsophagus. Each gland cell is pear-shaped or irregular and contains a cytoplasm which always takes up hæmatoxylin in hæmatoxylin-congo-red stain and a large nucleolated nucleus. In the young state the cytoplasm contains numerous irregularly shaped granules staining well with hæmatoxylin, but in the older state it appears to be vesicular. The efferent ducts from the gland cells proceed anteriorly forming a pretty large bundle on either side of the œsophagus, and open into the posterior end of the pharynx at the dorso-lateral side.

The œsophagus commences at the beginning of Segment III and reaches to the posterior septum of Segment V, the dorsal median inlet of the triradiate pharyngeal lumen being lost more or less suddenly at the beginning. In cross sections it is elliptical all through. Owing to the absence of the septum at the intersegment II/III, the alimentary canal of this portion is widest at the junction of the pharynx and the œsophagus narrowing towards both ends, so as to appear fusiform when seen from the dorsal side, but the œsophagus is distinctly set off from the pharynx by the disappearance of the triangular outline of the latter. Each cell of the œsophageal endoderm contains a compact, finely granular cytoplasm and a nucleolated nucleus at the proximal side. The outer peritoneum sometimes shows a cellular structure, especially on the dorsal side, and in Segment V some chloragogues are often developed.

The intestine begins at the anterior end of Segment VI, the external diameter of the alimentary canal being suddenly increased by the luxurious development of the outer chloragogue coat 100–120 μ thick. The intestinal lumen is nearly uniform in caliber in Segments VI and VII and is as wide as that of the œsophagus, but in Segments IX—XV it is very spacious, measuring about one-fourth to one-third of the segment width, and more posteriorly it gradually narrows to one-fifth or less. The endoderm consists of ciliated columnar cells with the nuclei lying at the middle. The thickness of the endoderm in Segments VI and VII is 30 μ or more, in the middle segments almost

20 μ, and in the posteriormost segments only 10 μ or less. The chloragogue coat is well developed also in Segments VII—IX, but the thickness decreases posteriorly, and in Segment X it is only 20–30 μ. The chloragogue coat is entirely absent from the middle and posterior segments. Each chloragogue cell has a more or less granulated cytoplasm and a nucleus containing a large nucleolus in the distal half.

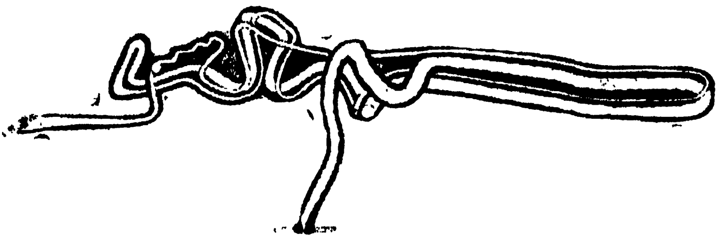
4. NEPHRIDIA.

There are two forms of nephridia. Each funnel always lies in the preceding segment, and is as usual ciliated around its mouth.

The anterior nephridia are present in pairs in Segments VII and VIII. The funnel is continued into a long narrow canal which immediately pierces the septum, and becoming smaller proceeds dorsalwards along the lateral curvature of the intestine. At the dorsal end it undergoes a few convolutions and then turns ventralwards entwining with the ascending canal and enters the main mass of the nephridium at the ventral side of the intestine. In the main mass the canal undergoes several convolutions and after leaving it at its posterior end, the canal proceeds in a wavy course to the nephridial pore, which opens just in front of the ventral setæ. The caliber of the lumen is nearly uniform, measuring about 5 μ, except at the short narrower proximal portion. The anterior nephridium often presents a racemose appearance because of the presence of large vesicular peritoneal cells. The shape of the main mass is discoidal or flat ellipsoidal.

The posterior nephridia begin with Segment XIII and are present only on the left side of the body. The funnel is continued into a narrow canal, which after piercing the septum soon enters the main mass (Fig. 10). This is long, somewhat undulate, 40–60 μ thick and lies always by the side of, and spreading over, the ventral vessel, to which it closely adheres. In most cases it reaches nearly to the posterior septum of a segment, but sometimes the posteriorly elongate portion turns back anteriad, and gives rise to a certain resemblance to an anterior nephridium. The proximal canal of each posterior nephridium has a caliber of 4–7 μ, and proceeds in a long wavy course to the posterior end of the main mass. Then turning and proceeding anteriorly it opens into a wider canal at about the anterior end of the main

Fig. 10.



Posterior nephridium from Segment XX of a well narcotized specimen.

mass. This canal measures 10–20 μ across and proceeds backwards also in a wavy course and again turning back at the posterior end, proceeds forwards to about the middle of the whole organ, where it leaves the main mass and runs to the left nearly straight to the posterior nephridial pore, lying at about one-third segment length from the anterior septum on the longitudinal, ventral setal line at the left side of the body.

The nephridia, both the anterior and the posterior, often show a temporary ampulla near the nephridial pore. The canal is not ciliated, neither is it furnished with a special ampulla, as is figured and precisely described by Dixon (1915) for Tubifex rivulorum.

The absence of nephridia from one side of the body has been noted in the following Tubificidæ.

Rhizodrilus lacteus Smith. " Nephridia first appear in VIII, but in VIII, IX and somites posterior to XI only one nephridium is present in each somite, and that is in the left side of the body in all cases observed " (Smith, 1900).

Tubifex irroratus (Verrill). " Nephridia are frequently developed on one side only or altogether absent from many somites " (Moore. 1905). This species accurately belongs to the genus Monopylephorus.

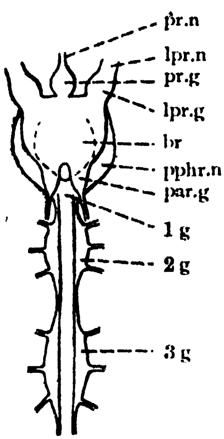
Tubifex hamatus Moore. " Nephridia are present on the left side only of most, if not of all segments " (Moore, 1905).

5. NERVOUS SYSTEM.

The brain lies near the dorsal wall of the body between the prostomium and Segment I. As shown above it is almost square, somewhat narrowed posteriorly and shallowly notched on the posterior border. Its cross section is always elliptical.

" The brain," writes Dixon (1915) for Tubifex rivulorum, " is concave behind and in front, and consists of a solid mass of nerve fibers and nerve cells. Anteriorly it is produced into three horns or lobes, two lateral and one median: the former may be described as the antero-lateral lobe, and the latter as the anterior median lobe.................The antero-lateral lobes described above are not figured by Vejdovsky (1884) in his drawing of the brain of Tubifex rivulorum, and he definitely states that the brain in this region is devoid of processes or lobes. He figures, however, postero-lateral lobes from which well-developed muscles pass to the body wall, and these lobes can be clearly seen, but I have never been able to find the muscles. Beddard described, in his definition of this species, a median, smaller, posterior lobe corresponding to the anterior one; but this I have never been able to see."

Fig. 11.



Brain and anterior portion of ventral nerve cord; reconstructed. br—brain, 1, 2, 3 g—first, second and third ventral ganglia, lpr.g—lateral prostomial ganglion, lpr.n—lateral prostomial nerve, par.g—parietal ganglion, pr.g—prostomial ganglion, pr.n—prostomial nerve, pphr.n—peripharyngeal nerve.

In Tubifex hattai five ganglia are also attached directly to the brain (Fig. 11): three on the anterior border and two on the posterior. The prostomial ganglion or anterior median lobe is situated at the middle of the anterior border and the paired, lateral prostomial ganglia or antero-lateral lobes on the antero-lateral corners of the brain; the paired parietal ganglia or postero-lateral lobes are attached to the posterior border. All of these ganglia are seemingly stouter than those figured by Dixon.

The prostomial nerve starts from the anterior end of the prostomial ganglion and sends out diverging branches into the prostomial wall.

The lateral prostomial nerve is somewhat strong and runs out from the lateral prostomial ganglion on either side of the brain obliquely outwards, and after a short course enters the lateral wall of the prostomium and becomes the lateral line of the body.

The parietal nerve runs out from the parietal ganglion on either side of the brain obliquely backwards to the dorsal body wall. Some of its branches may enter the pharyngeal wall, but on this point I am not certain. This nerve is neither figured nor described by Dixon (1915) for Tubifex rivulorum, and she denies even the presence of muscle fibers, which are figured by Vejdovsky (1884). In Limnodrilus gotoi (Nomura, 1913) such muscles are well developed, but in Tubifex hattai also I have never been able to find them.

The peripharyngeal nerve runs out from the latero-ventral border on either side of the brain directly behind the lateral prostomial ganglion and proceeds downwards along the curvature of the body wall, and uniting with its fellow of the opposite side on the ventral median wall of Segment I forms the first ventral ganglion.

The ventral nerve cord is very slender and measures 10–20 μ across, excluding the ganglionic enlargements.

The ventral ganglion of each segment anterior to V occupies about the whole length of the segment, but in each posterior segment it is always confined to the posterior half. The broadest portion of each ventral ganglion in the anterior segments measures generally 80 μ across, while in the posteriormost segments it is less than 40 μ.

The median neural canal is found only in Segment V. If the lateral neural canals are present, they must be of very feeble development. The musculature of the nerve cord is of the usual type for Tubificidæ.

6. VASCULAR SYSTEM.

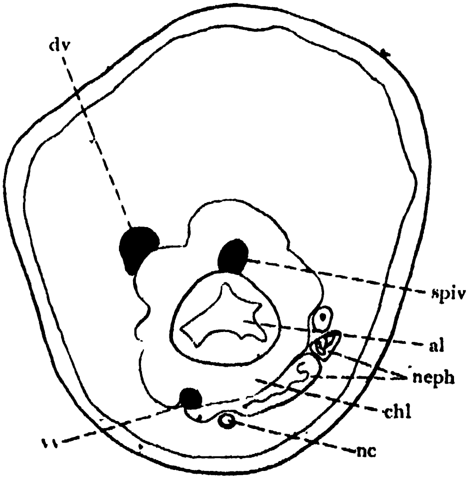
The longitudinal trunk of the dorsal vascular system consists of the dorsal vessel, the supraintestinal vessel and the dorso-supraintestinal vessel. The ventral vascular system is somewhat complicated by the discontinuation of the ventral vessel in Segments VIII and IX and by the presence of segmentary subintestinal vessels.

1) The dorsal vessel commences directly under the prostomial ganglion. Running backwards directly under the brain the vessel passes through its posterior notch between the parietal ganglia and approaches nearer until it again lies very near to the dorsal body wall. It then turns toward the posterior end of the pharynx and lies between the posterior pharyngeal glands in Segment III. In Segments IV and

V it lies near the dorsal median line of the œsophagus. At the beginning of Segment VI, where the chloragogue coat is abruptly developed, the vessel swells slightly and turns suddenly dorsalwards to lie for a while on the dorsal side of the intestine. In Segments VI and VII the vessel runs for the greater part of its course on the left side of the intestine, invariably resuming its normal dorsal position at the intersegments (Figs. 5 and 6 dv). In Segment VIII it at first runs just as in the preceding segment (Fig. 12 dv), but in the posterior part of the segment, where the intestinal hearts are given off from the supraintestinal vessel, it somewhat suddenly resumes its normal position (Fig. 13 dv) and runs to the end of the segment on the dorsal side of the intestine. In Segment IX starting from its normal position and attached to the chloragogue coat it turns toward the right side of the intestine (Fig. 14 dv) and unites at last with the supraintestinal vessel to form thereafter the dorso-supraintestinal vessel.

2) The 'dorso-supraintestinal' vessel is a new name which is proposed by myself, in this sense that the vessel is formed theoretically

Fig. 12.



Cross section through the anterior part of Segment VIII. al—alimentary canal, chl—chloragogues, dv—dorsal vessel, nc—ventral nerve cord, neph—anterior nephridium, spiv—supraintestinal vessel, vv—ventral vessel.

by the union of the dorsal and supraintestinal vessels, and this is nothing but the greater posterior portion of the 'dorsal' vessel as hitherto commonly named.

This vessel begins at about the middle of Segment IX and is attached directly to the intestinal endoderm. In most segments behind X the vessel runs for the greater part of its course along the right side of the intestine (Figs. 3, 4 and 16 dv), always resuming its normal dorsal position at each intersegment. The position of the dorso-supraintestinal vessel in the right side in the middle part of a segment is in my opinion due to a displacement of the intestine within each segment. In the posteriormost segments it runs always on the dorsal side of the intestine (Fig. 15 dv). The caliber of the vessel is rather greater in postclitellar than in preclitellar segments.

3) The supraintestinal vessel is not well enough developed to be pointed out in the segments anterior to VI, but further backwards it is well developed on the dorsal side of the intestine, attached closely to the endoderm. It runs in the dorsal median line of the intestine to Segment IX (Figs. 12, 13 and 14 spiv), giving off the intestinal hearts at the posterior septum of Segment VIII. In Segment IX it lies at first in the dorsal median line, irrespectively of the position of the dorsal vessel, but gradually shifting to the right side of the intestine at last unites with the dorsal vessel (Fig. 14 spiv). In Tubifex rivulorum Dixon (1915) could not find the opening of this vessel into the dorsal vessel and states that "the vessel seems rather to diminish gradually in size, and finally to disappear."

4) The subintestinal vessel can not be distinguished in segments anterior to VI owing to the too feeble development of the intestinal vascular network, but behind Segment VI it forms gradually and can easily be made out in the ventral median line of the intestine closely attached to the endoderm and thickly covered by the chloragogue coat, in Segments VII and VIII uniting with the ventral vessel (Figs. 13 and 14 ssbiv). In Segments IX—XI a true subintestinal vessel is present apart from the ventral vessel, lying for the greater part of a segment length on the left side of the intestine, which is dislocated in the middle of each segment (Figs. 3, 4 siv and 14 sbiv). The vessel opens into the ventral vessel just in front of the posterior septum of Segment XI.

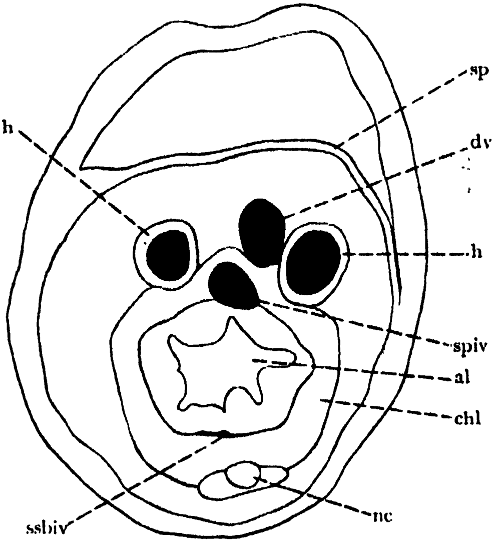
5) The ventral vessel runs through the whole body length and for the greater part of its course it lies along the ventral side of the alimentary canal a little to the left side of the ventral nerve cord; but as the vessel is discontinued in Segments VIII and IX by the atrophy of the intermediate ventral vessel, it is more convenient to distinguish the vessel into an anterior and a posterior part. According to the figures and descriptions of Vejdovsky (1884), Dixon (1915) and Meyer (1917), the atrophy of the intermediate ventral vessel is not the case in Tubifex rivulorum.

The anterior ventral vessel commences at the anteriormost end of the dorsal vessel, which is here divided into two lateral vessels. Each of these paired ventral vessels runs backwards along the lateral wall of the buccal cavity and the pharynx, and gradually proceeding ventralwards and backwards dorsal to the ventral nerve cord, it unites to form the median ventral vessel just in front of the first septum (III/IV). In Segments IV and V the ventral vessel proceeds backwards through the interspaces of the posterior pharyngeal glands on the ventral side of the intestine. In Segment VI it is often attached to the chloragogue coat. In Segments VII and VIII, where it unites with the subintestinal vessel, it is always attached to the endoderm (Fig. 13 ssbiv), except in the neighborhood of the septa (Figs. 6 and 12 vv). In the posterior region of Segment VIII it is continued as the subintestinal vessel (Fig. 14 av—sbiv).

The posterior ventral vessel begins where the intestinal hearts of the two sides are united (Fig. 14 vv and Figs. 3, 4, 15 and 16 vv).

6) The intestinal hearts (Figs. 13 and 14 h) lie in Segment VIII and originate from the supraintestinal vessel where Septum VIII/IX starts from the dorsal body wall. Soon after its origin the heart suddenly increases its caliber and running laterally for a little while on the outside of the chloragogue coat, turns posteriorly and proceeds backward on the dorso-lateral side of the intestine. On reaching the posterior septum, it makes a sudden ventral turn and runs along the curvature of the intestine, gradually diminishing in caliber. On approaching the ventral nerve cord it again turns posteriorly and penetrating the septal sac proceeds into Segment IX. The two hearts, after running side by side in Segment IX, at last unite at the commencement of the posterior ventral vessel.

Fig. 13.

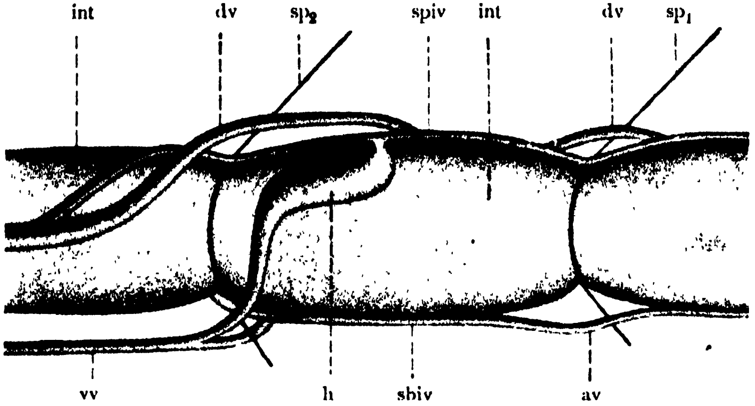


Cross section through the posterior part of Segment VIII. al—alimentary canal, chl—chloragogues, dv—dorsal vessel, h—heart, nc—ventral nerve cord, ssbiv—subintestinal vessel, sp—Septum VIII/IX, spiv—supraintestinal vessel.

The wall of the heart is thick compared with that of the ordinary vessel, and consists of five layers. The outermost layer is that of the cellular peritoneum which is followed by a layer of elastic fibers and inside of this comes the circular muscle layer, the longitudinal muscle layer and the intima one after the other. The structure of the layer of longitudinal muscle fibers of this species is quite different from that of Japanese Limnodrilus, each fibers standing apart instead of forming bundles as in the latter. The layer of elastic fibers and the peritoneum are interrupted by cells which may possibly be nervous in nature and are arranged in a continuous line on the extero-lateral surface of the heart. Each of these cells contains a large nucleolated nucleus and a dense cytoplasm. In the distal half of the heart the layers of elastic and longitudinal muscle fibers are wanting.

7) The cœlomic commissures are generally very fine except those in the anteriormost segments, but they never give out branches to

Fig 14.



Diagrammatic representation of heart and neighboring blood-vessels. av—anterior ventral vessel united with subintestinal vessel, dv—dorsal vessel, h—heart, int—intestinal wall, sbiv—subintestinal vessel, sp_{1-2}—Septa VII/VIII and VIII/IX, spiv—supraintestinal vessel, vv—posterior ventral vessel.

form anastomoses.

In Segment I the commissures are represented by the paired ventral vessels which are direct continuations of the dorsal vessel.

In Segments II and III the commissures are given off from the dorsal vessel in the corresponding setal plane and after a few windings they open into the paired ventral vessels in the same plane.

In Segments IV—VII they always start from the dorsal vessel just in front of the posterior septum of each segment and after several windings enter the median ventral vessel in the setal plane.

The commissures in Segments III—V are entangled in the pharyngeal glands.

In Segment VIII they are absent.

In Segments IX—XI they branch off from the dorso-supraintestinal vessel just in front of the posterior septum of each segment and after several windings enter the ventral vessel also just in front of the septum. All of them pulsate rhythmically. In young specimens the commissures of Segment IX are less developed and look like commissural hearts, but in sexually mature ones they are very well developed and surround the outer surface of the anterior sperm-sac. In Segment X soon after starting from the dorso-supraintestinal vessel they enter the posterior sperm-sac (Fig. 4 cv) and emerging therefrom they undergo a few windings (Fig. 3 cv) before they open into the ventral vessel. In Segment XI they enter the ovisac and undergo several windings as they course between the sperm-ducts and the alimentary canal.

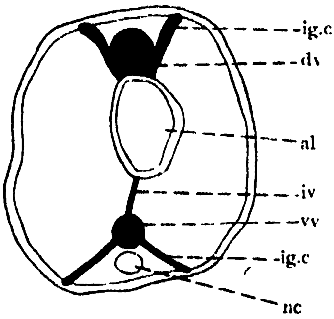
In segments posterior to XII they start just in front of the septum from the dorso-supraintestinal vessel, but the openings into the ventral vessel gradually shift forwards to about the middle between the setal plane and the posterior septum.

In each posteriormost segment the starting point from the dorso-supraintestinal vessel also lies forwards in the same plane as the ventral termination (Fig. 15 ig. c).

In Segments II—V and IX—XI the commissures are cœlomic and in Segments VI, VII and XII— they are integumentary.

In Segments VI, VII and IX—XI the commissures run in an undulating dorso-ventral course mostly in the posterior half of a segment, but in the postclitellar segments they follow a wavy antero-posterior course through the whole segment length.

Fig. 15.



Schematized cross section through a segment near the posterior end of the body to show the relation between the blood-vessels. al—alimentary canal, dv—dorso-supraintestinal vessel, ig. c—integumentary commissure, iv—intestinal vessel, nc ventral nerve cord, vv— ventral vessel.

8) The intestinal networks are not so luxuriously developed as in some other species and in most segments, especially behind the clitellum they rather form lattice-work, though sometimes a few connectives between the bars are found. The number of bars in a segment is indefinite.

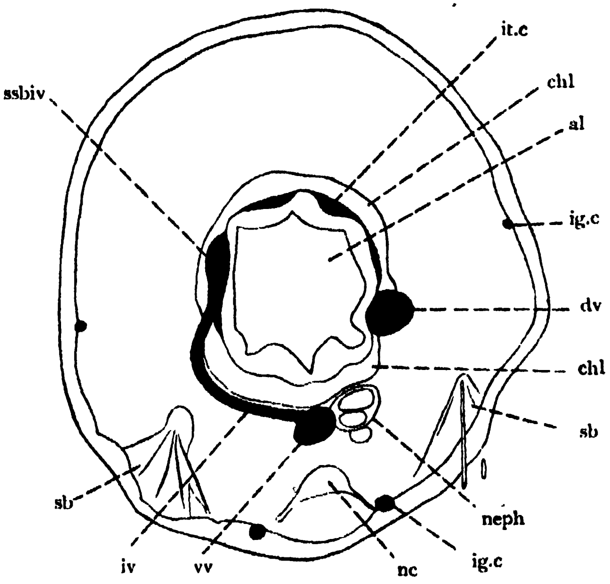
In Segments II—IV the intestinal networks are very feebly developed and directly connect the supraintestinal and the subintestinal vessels.

In Segments VII and VIII they are more or less well developed.

The bars are started out directly from the supraintestinal vessel which lies in the dorsal median line and open into the subintestinal vessel in the ventral median line of the intestine where the latter vessel is united with the ventral vessel. The networks are equally developed on both sides of the intestine.

In the anterior half of Segment IX they show the same arrangement as in the preceding segment.

Fig. 16.



Schematized cross section through a middle segment to show the relation between the blood-vessels. al—alimentary canal, chl—chloragogues, dv—dorso-supraintestinal vessel, ig. c—integumentary commissure, it. c—intestinal network, iv—intestinal vessel, nc—ventral nerve cord, neph—posterior nephridium, sb—seta-bundle, ssbiv—segmentary subintestinal vessel, vv—ventral vessel.

In the posterior half of Segment IX and in Segments X and XI the bars of the intestinal lattice-works branch off directly from the dorso-supraintestinal vessel which lies on the right side of the intestine, and open separately into the subintestinal vessel on the left side of the intestine. Behind these segments the intestinal lattice-works are well developed only on the dorsal side of the intestine (Fig. 16 it.c), that is on the left side in the normal position.

In Segments posterior to XII the bars invariably start from the dorso-supraintestinal vessel, and on reaching the left side of the intestine they open into the segmentary subintestinal vessel in each segment. The segmentary subintestinal vessel is a longitudinal vessel confined to one segment only and lies on the ventral side of the intestine in the normal position. If these are continued in one longitudinal vessel through many succeeding segments, it is, of course, to be called the subintestinal vessel. The segmentary subintestinal vessel communicates with the ventral vessel by the intestinal vessel which lies in the setal plane (Fig. 16 iv).

In each segment more posterior to these the segmentary subintestinal vessel disappears leaving the intestinal vessel alone (Fig. 15 iv)

In the posteriormost segments both the intestinal vessel and the intestinal lattice-work are wanting.

9) The blood corpuscles are often found in the anterior portion of the main longitudinal vessels. They are spherical and contain each a homogeneous cytoplasm and a compact nucleus.

7. GENITAL SYSTEM.

1) Testes. There are one pair attached to the posterior face of Septum IX/X. Each testis originates at a point which lies between the ventral nerve cord and the longitudinal ventral setal line and bulges out into the cœlom of Segment X on the outer side of the septal sac.

2) Sperm-sacs. Two sperm-sacs are found: an anterior and a posterior. Both are outgrowths of the septa of Segment X and lie always in the dorsal region of the body. The anterior sperm-sac originates from the anterior septum and lies in Segment IX, while the posterior one is formed by the posterior septum and extends backwards into Segment XIII; the wall is very thin and structureless.

In young specimens the anterior sperm-sac is not formed even when the posterior sperm-sac is conspicuous in Segment XI. The young anterior sperm-sac shows absolutely the same structure with the intersegmental organ and in some specimens it is directed backwards into Segment X instead of bulging out into Segment IX. These facts appear to me to show that the sperm-sac are homologous with the intersegmental organs and also that the directions in which they grow out are influenced solely by the mechanical pressure of the cœlomic fluid.

"As it (sperm-sac) increases in size," writes Dixon (1915) for Tubifex rivulorum, "its structure becomes more complicated. The cavity becomes divided up into a series of much smaller spaces by the growth inwards of its wall. These small spaces remain in communication with one another, and are filled with spermatozoa in all stages of development." Something similar may occur also in Tubifex hattai, but at any rate it is not so remarkable as is described by Dixon.

3) Sperm-ducts. Each sperm-duct consists of a wide funnel, a very long vas deferens, a short strium, an irregularly shaped prostate gland, a penis and a penial chamber.

a. Funnel. The funnel opening in Segment X is attached to the anterior face of Septum X/XI near its bottom, and as the septal wall here runs backwards for a while parallel with and on either side of the intestine, so the funnel is situated in nearly the same plane directed just inwards. It is rather discoidal and widely open. The wall of the funnel consists of two layers. The inner layer consists of regularly arranged, ciliated columnar cells of two different heights, the central cells being much taller than the peripheral. The central cells are very vesicular and contain a feebly staining cytoplasm forming strands vertical to the wall, and a nucleus near the proximal base. The peripheral cells contain a dense cytoplasm also forming strands, and a nucleus near the middle. The cilia are all of nearly equal lengths. The outer layer is formed by the connective tissue derived from the peritoneum.

b. Vas deferens. The funnel is continued as a very long vas deferens which is circular in cross section and when seen in *toto* striated transversely all through. The vas deferens is attached to the septum only at its beginning; the remainder lies free in the cœlom, and consequently assumes different positions between other organs and very often enters the ovisac. In its narrow proximal portion it is ciliated and the distal portion is wide and non-ciliated. The lengths of the two portions are nearly equal. In living material the ciliary motion is always visible in the proximal portion but in the non-ciliated portion there is no motion. In both portions the wall consists of two layers; an inner layer differing in structure in its different portions, and an outer layer formed by a thin peritoneal connective tissue containing regularly arranged, fine longitudinal muscle fibers.

The ciliated portion commences directly behind the funnel where the central cells somewhat suddenly give place to more flattened ones and the cilia are directed backwards. The flattened inner epithelial cells show no peculiarities at the beginning, but soon assume the form of a ring, the central lumen of which forms the cavity of the duct. In a longitudinal section of the tube the inner surface of a ring cell is flat, and the outer convex, so that if the section passes through the wall tangentially these cells closely resemble striated muscle fibers. The transverse striation of the ciliated portion is therefore external to the tube. The cytoplasm is compact and uniform. The nucleus is compact, elongate and curved around the lumen, but it is not so long as is described and figured by Dixon (1915) for Tubifex rivulorum. "Each cell (flat, annular or ring-like cell)," writes Dixon, "is perforated in the center by a large, rounded lumen, about 42 μ in diameter, the wall around it being comparatively thin." In Tubifex hattai, the outer diameter of the tube is 25 μ all through, except at the beginning which is wider and has a wall 5 μ thick.

The ciliated portion at first runs straight backwards and on reaching about the middle of Segment XI it undergoes several windings and then opens into the wider, non-ciliated portion. The transition of the two portions is somewhat sudden. The cilia and the outer convexity of a ring cell disappear first, and the compact, uniform cytoplasm becomes rich in strands running perpendicularly to the wall, leaving a compact cytoplasmic layer at the inner surface of the lumen, and then the wall thickens, the nucleus becomes spherical and at last the lumen becomes surrounded by two or three crescent shaped cells instead of one ring cell. "They (epithelial cells)," writes Dixon (1915) for Tubifex rivulorum, "are ring-shaped, piled one on top of the other extremely regularly, and each one is perforated in the center by a rounded lumen. Each cell has its greatest thickness around the lumen of the tube, and tapers off considerably at its outer edge. The nuclei of these cells resemble very closely those of the first part (ciliated portion) of the tube, and are situated near the inner edges of the cells. The greater thickness of the wall of this part of the tube is due to a considerable increase in the quantity of the matrix in which the cells are imbedded, and by which they are surrounded. This matrix still stains only with plasma-stains, but it is no longer structureless, for it is characterized by the formation in it of a large number of exceedingly fine fibrillæ." According to this description the minute structure of the non-ciliated portion of Tubifex hattai totally differs from that of Tubifex rivulorum. The lining cytoplasmic layer of the lumen always makes regular wavy foldings which give rise to transverse striation of the tube. The outer diameter of the portion differs according to specimens; in some cases it is about twice times as thick as the ciliated portion, its caliber remaining constant, and in other cases it is three or four times as thick with increased caliber.

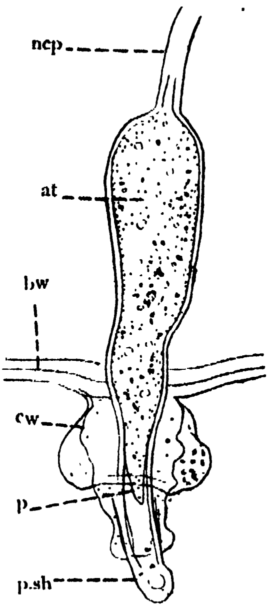
c. Atrium. This is a conical or rather bat-shaped organ into which the non-ciliated portion of the vas deferens opens excentrically at its wider proximal end, and after gradually narrowing and undergoing a few slight windings leads to the penis at the distal end. The widest part of the organ at the proximal end is more than two or three times as wide as the non-ciliated portion and into it open the lobe canals of the prostate gland through an aperture which lies mostly on the ventral surface of the organ. The atrium is also circular in cross section. The organ in a well narcotized specimen is longer than half of the segment length. The atrial wall consists of three layers. The outer layer is very thin and is the continuation of the connective tissue of the vas deferens, but it becomes very thick and cellular near the distal end. The middle layer is well developed and consists of circular muscle fibers; the longitudinal muscle fibers appear to be absent. The innermost layer consists of glandular cells, the crescent shaped cells of the non-ciliated portion suddenly giving place to cubical, glandular ones at the beginning of the atrium. On the ventral side of the atrium where it receives the vas deferens as well as the prostate gland, the wall is much thickened by the convergent ducts of the prostate gland finding their way into the interspace between the epithelium and the muscle layer, and causing the lumen to become crescent-shaped in cross section at this portion. Some gland cells are very vesicular and some contain numerous fine, spherical granules of the same size which stain very well with eosin, but not with carmine or congo-red. The nuclei are nucleolated and situated at the proximal side. The structure of the proximal half of the atrium well resembles that of Limnodrilus gotoi and can not be distinguished from it in sections stained with carmine, unless other organs are taken into consideration.

d. Prostate gland. It is of irregular shape in full grown specimens and surrounds the atrium more or less on its ventro-lateral side. The general aspect of the organ is of the usual type seen in higher Tubificidæ. Most of the cells are of irregular or pear shape and the cytoplasm is sometimes vesicular and sometimes finely granular. The lobe canals, sometimes uniting with others, open into the atrium as stated above, and in their lumen are found many fine granules which are possibly secretions from the prostate gland cells. These granules as well as the granules found in the prostate gland cells stain exactly the same as those found in the atrial cells with eosin, congo-red or carmine.

e. Penis. It is the direct prolongation of the atrial epithelium enclosed in a cellular sheath which is the continuation of the lining wall of the penial chamber formed by the invagination of the body wall. The penis as commonly described has a double wall, the inner layer being the direct continuation of the atrial wall and the outer of the lining of the penial chamber; but in the present species the penis is formed only by the continuation of the atrial epithelium. The penis proper is conical in shape and about 50 μ long. Its wall consists of irregularly shaped cells containing a vesicular cytoplasm and the lumen is consequently somewhat irregular in form.

Whether the penis is protruded (Fig. 17 p. sh) or retracted (Fig. 18 ew and iw) its cellular sheath always protects it. The sheath is strongly constricted at its proximal end and consists of two distinct walls, whose

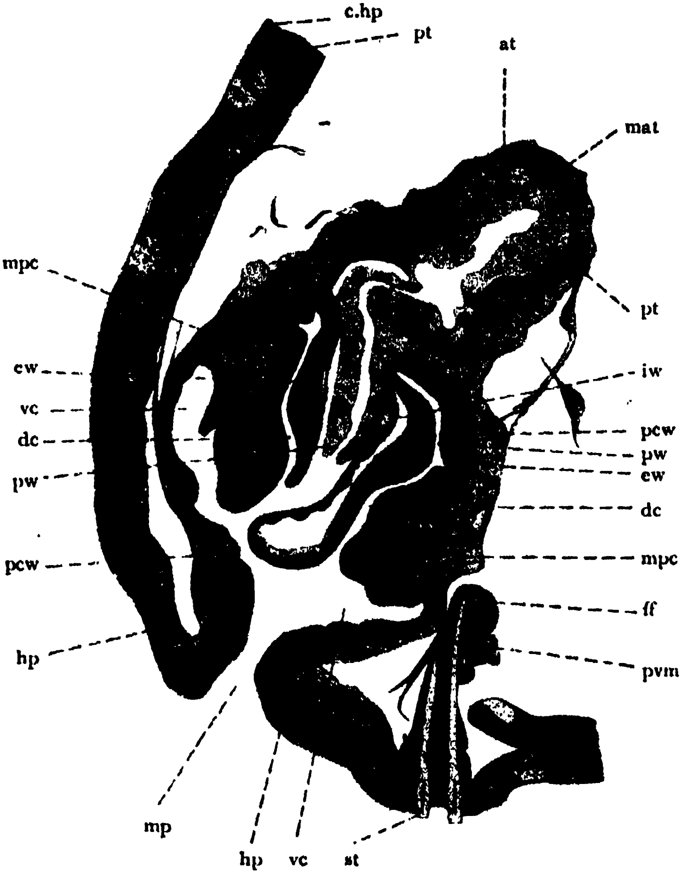
Fig. 17.



Atrium, penis and penis sheath from a living narcotized specimens; penis protruded by compression. at—atrium, bw—body wall, cw—everted wall of penial chamber, ncp—non-ciliated portion of vas deferens, p—penis, p.sh—penis sheath.

epithelial cells are distinguished by a more compact cytoplasm when compared with those of the penial wall. The inner wall begins at the proximal base of the penis proper and, extending downwards past

Fig. 18.



Longitudinal section of penis and penial chamber (from a cross section of the body). at—atrium, c. hp—clitellar hypodermis, dc—dorsal cavity of penial chamber, ew—outer wall of penis sheath, ff—follicular fundus of setigerous organ, hp—hypodermis of extraclitellar structure, iw—inner wall of penis sheath, mat—atrial muscle, mp—male pore of the left side of the body, mpc—muscular layer of the wall of penial chamber, pcw—wall of penial chamber, pt—peritoneum, pvm—parietovaginal muscle, pw—penial wall, st—ventral setæ, vc—ventral cavity of penial chamber.

its apex, is continued into the outer wall which extends upwards to the proximal end of the penis, showing a marked thickning at the middle of its length. The lip of the sheath is longer on the inner side and presents here a hollow swelling to make the end obtuse and smooth. The inner wall of the sheath is very thin and consists of two layers, the outer hypodermis and the inner peritoneum. The outer wall consists of four layers, an outermost of very thin but visible cuticle, followed by the hypodermis, the regularly arranged fine, circular muscle fibers and the peritoneum.

As to the penis of Tubifex rivulorum (Tubifex tubifex) different descriptions are given by following investigators.

Ditlevsen (1904). " Sie entspricht in allem wesentlichen der Beschreibung von Nasse; nur finden sich hier nicht drei Hauttrichter, sondern nur zwei, ein äusserer und ein innerer.................sie (Cuticula) ist an der Spitze des Penis mit kleinen Dornen versehen, ist leicht braun gefärbt..................."

Pointner (1911). "Die einzelnen Teile des Penis sind folgende: 1) Der äussere Copulationstrichter (=? greater, ventral wall of the penial chamber in my description)..............2) Das Präputium (=? smaller, dorsal wall of the penial chamber)...............3) Die Glans penis (=the thickened portion of the outer wall of the penis sheath). 4) Die auf die Glans penis folgende dünne Penismembran (=whole inner wall and distal thinner portion of the outer wall of the penis sheath) und 5) Die Penisspitze (= penis) mit der äusseren, seitlich gelegenen Öffnung. Von diesen Abschnitten hat Vejdovsky (1884) die vier zuerst genannten beobachtet, den fünften hingegen scheint er wenigstens nicht als besonderen, ausstülpbaren Teil erkannt zu haben...............an dem äusseren Rande (der Penismembran) zehn zarten, schwach braun gefärbten Zähnchen versehen ist. (Diese Zähnchen sind, was Zahl und Grösse anbelangt, sehr verschieden und können, wie es scheint, auch ganz fehlen.)"

Dixon (1915). "The structure of the penis is somewhat complicated. Its cavity is continuous with that of the (spermiducal=atrial) gland, and it is surrounded by a penis sheath, which, when the penis is retracted, is double, the outer layer being continuous with the epidermis of the body wall. We may, therefore, speak of the outer and inner penial sheaths, both consisting of a single layer of epithelial cells, somewhat cubical in shape and provided with conspicuous, rounded, nucleolated nuclei. The cuticular secretion, which forms a delicate layer outside the cells of the epidermis, is continued over the cells of the penial sheath, and in the retracted condition of the penis may completely fill the space between the outer and inner penial sheaths. ....The cavity of the penis proper is surrounded by a single layer of elongated cells which encroach considerably on the lumen. Near the apex of the penis they are so large that they almost obliterate the lumen altogether."

Since I have made no direct observations on the structure of the penis and penis sheath of Tubifex hattai at several stages of development, I can not decisively comment on the difference of the descriptions cited above whether it depends upon the difference of the developmental stages or of species. At any rate in Tubifex hattai the structure of the penis and penis sheath mentioned above is a constant one at least in adult specimens which are usually collected and the parts are exactly identical with those observed by Pointner. In the comparison of his Text-figures 1 and 2 with my Figures 17 and 18, however, a distinct difference can be found between them; that is, in Tubifex rivulorum the penis can protrude or always protrudes outside the distal end of the penis sheath, while in Tubifex hattai the former is constantly covered and protected by the latter. The cuticular processes described by Ditlevsen and Pointner I have never been able to see.

f. Penial chamber. It is naturally divided into a dorsal and a ventral cavity. The dorsal one lies between the outer wall of the penis sheath and the dorsal portion of the penial chamber and is almost cylindrical as a whole. Its wall consists of five layers, the same as those of the body wall. The ventral cavity extends horizontally below the penis sheath and is almost circular in outline; the muscle layer of its wall is very poorly developed. The male pore opens just outside the ventral setæ.

4) Spermathecæ. One pair of spermathecæ are present in the dorsal side of Segment X. Each one consists of two portions: a pear-shaped, saccular portion and a cylindrical, duct portion which receives the narrow end of the former and leads to the external opening. The opening (Fig. 3 spth.p) lies directly dorsal to the lateral line on either side of the body in the setal plane of segment X. It is stated by Dixon (1915) that in Tubifex rivulorum the duct

portion opens into the exterior near the ventral setæ of Segment X.

When the worm is young and the organs are still small, they are always arranged symmetrically, undergoing a sigmoid contortion with the blind end directed anteriorly, and the duct portion is longer than half of the saccular. In full grown specimens, however, the saccular portion is unusually distended and three times as long as the duct portion and more than half as wide as the segment and longer than it, so that enough space can not be found for the pair in a segment. The result is that sometimes one extends into the anterior sperm-sac and the other into the posterior sperm-sac or one may be twisted around the narrow part of the other.

The thickness of the wall of the saccular portion differs notably according to age. When fully distended it is only 10μ, but in young specimens it is about 50μ. The wall consists apparently of two layers. The inner epithelium mostly presents a smooth internal surface and consists of flat or cubical cells mostly without any visible boundaries, and with mostly spherical nuclei, situated at the middle and containing two nucleoli. The cytoplasm of the epithelium appears homogeneous when stained with eosin, but finely granulated, especially in the distal half, when stained with congo-red. The outer layer is the thin peritoneal connective tissue containing two feebly developed layers of muscle fibers: the outer one of longitudinal and the inner of circular, both fibers running rectangular with each other.

The duct portion measures 50 μ or more across and 15–20 μ in caliber and is circular in cross section all through. The epithelium consists of cubical or columnar cells and is about 10 μ thick. The inner surface is ciliated and each cell contains a cytoplasm usually forming strands, and a compact nucleus at the middle. The outermost peritoneum, the longitudinal and the circular muscle fibers are all well developed. Near the end of the duct portion the wall becomes irregularly folded, the cilia and the cytoplasmic strands disappear and a cuticle comes to view. The presence of the ciliated epithelium of the duct portion is also described by Dixon (1915) for Tubifex rivulorum.

Spermatophores are always present in the saccular portion.

5) Ovaries. One pair of ovaries are attached to the posterior face of Septum X/XI on either side of the nerve cord ventral to it.

6) Ovisac. This is a posteriorly directed outgrowth of Septum

XI/XII and lies on the dorsal side of the intestine reaching to Segment XIII as far as I have observed.

7) Oviducts. One pair of oviducts lie in the intersegment XI/XII, in the ventral setal lines. The duct is very short and the funnel opens widely into the cœlom of Segment XI.

LITERATURE.

1) BEDDARD, F. E., 1895 A monograph of the order Oligochæta.

2) DITLEVSEN, A., 1904 Studien an Oligochæten. Zeit. wiss. Zool., Bd. 77.

3) DIXON, G. C., 1915 Tubifex. L. M. B. C. Memoirs, XXIII.

4) EISEN, G., 1885 Oligochætological researches. Ann. Rep. Com. Fish and Fisheries for 1883.

5) MEYER, F., 1917 Untersuchungen über den Bau and die Entwicklung des Blutgefässsystem bei Tubifex tubifex (Müll.). Jena. Zeit., Bd. 54.

6) MICHAELSEN, W., 1900 Oligochæta (Das Tierreich).

7) MOORE, J. P., 1905 Some marine Oligochæta of New England. Proc. Acad. Nat. Sc. Philadelphia, LVII.

8) NOMURA, E., 1913 On two species of aquatic Oligochæta, Limnodrilus gotoi Hatai and Limnodrilus willeyi, n. sp. Journ. Coll. Sc. Imp. Univ. Tokyo., Vol. XXXV. Art. 4.

9) POINTNER, H., 1913 Beiträge zur Kenntnis der Oligochætenfauna der Gewässer von Graz. Zeit. wiss. Zool., Bd. 98.

10) SMITH, F., 1900 Notes on species of North American Oligochæta. III. List of species found in Illinois and descriptions of Illinois Tublificidæ. Bull. Ill. Lab. Nat. Hist., Vol. 5, Art. X.

11) STEPHENSON, J., 1922 On the septal and pharyngeal glands of the Microdrili (Oligochæta). Trans. Roy. Soc. Edinburgh, Vol. LIII.

12 VEJDOVSKY, F., 1884 System und Morphologie der Oligochæten.

ON THE SEX RATIO IN CARASSIUS AURATUS.

BY

KIICHIRO SASAKI.

Concerning the normal sex ratio in the vertebrates, we find the following relation. (Table I.)

TABLE I.

Order	Species	No. of ♂ : 100 ♀	No. of specimen	Authority
Mammalia	×Swine	111.8		
	×Oxen	107.3		
	Albino rat	106.46	607	King, H. D. & Cuénot, L. ('11)
	Albino rat	105.7	11175	King, H. D. ('24)
	Albino rat×wild Nor. rat	119.09	425	King, H. D. ('11)
	Norway rat	85.8	1862	King, H. D. ('24)
	×Rabbit	104.6		
	×Sheep	97.7		
	×Horse	99.7		
Aves	×Pigeon	115.0		
	×Hen fowl	94.7		
Pisces	×Cottus	188.0		
	×Lophius	385.0		
	Trout	92.4	488	Huxley, J. S. & Mrsic. ('23)
	Gambusia	11.1–12.5		Hildebrand, S. F. ('17)
	Gambusia (young)	95.8	94	Geiser, S. W. ('24)
	Lebistes reticulatus	100	2070	Henn, A. W. ('16)
	Aplochellus latipes	98.44	4838	Aida, T. ('24?)
	Cymatogaster	app. equal		Eigenmann, C. H. ('96)
	Spinax	app. equal		Punnett. ('04)
	Amphigonopterus	app. equal		Hubbs, C. L. ('21)
	Gray snapper	87.49	45	Hatai, S. ('18)
	Smooth dog-fish	78.98	315	Kellicott, W. E. ('08)
	*Amblygaster melanostictum	85.19	50	Sasaki, K.

*Ditrema temmincki	66.67	30	Sasaki, K.
*Salangichthys ishikawae	70,71	293	Sasaki, K.
*Hypomesus japonicus	192.31	38	Sasaki, K.
*Oncorhynchus keta	96.95	16477	Sasaki, K.
*Gold-fish (wakin)	100.00	30	Sasaki, K.
*Gold-fish (riukin)	87,18	73	Sasaki, K.
*Scomber japonicus	85.9	252	Sasaki, K.

×Goldschmidt, R.: Mechanism and physiology ef sex determination.
*Data not yet published.

As will be seen from Table I, the normal sex ratio is approximately 100♂ : 100♀, but the sex ratio in Carassius, which I am going to report now, is remarkable, the female being greatly in excess. This interesting fact was noted in 1923, when I was studying certain other problems about Carassius. For that investigation I collected Carassius of all sizes, from ponds and brooks near Masuda and Iwanuma Town, not far from the laboratory in Sendai. The total number of Carassius examined was 285. The smallest was 2 cm. in length and the largest 15 cm., but more than half of these were from 6 to 9 cm. long. The result of examination showed the ratio of 14.9 males to 100 females (See Table III).

This numerical relation between the sexes is certainly unique, but for definite statement concerning the sex ratio in Carassius still larger numbers must be examined, because I found afterwards that the sex ratio is variable not only according to the body length, but also according to the place where the fish live and the season when they are captured.

LIFE HISTORY OF CARASSIUS.

For the benefit of the reader who is not familiar with this interesting fish, the following brief note concerning their habitat, etc, may not be at all superfluous.

Carassius is a very common fish in Japan and China and is found everywhere in rivers, brooks, pools, and lakes of freshwater, though it may enter brackish water too. Carassius prefers water which is somewhat warm and rich in organic substances to cold, clear water. The fish which live in large ponds or lakes are of larger size an occassionally reach as much as 30 cm. in length, but those which live in the smaller brooks and pools are usually smaller in size, measuring from 5 to 6 cm. in length, and even the largest does not exceed 11 or 12 cm. The fish which live in clear water are usually silverly white along the abdomen, and are popularly called "Silver funa", while those living in slightly muddy pools or lakes are golden in colour and are called "Gold funa." Carassius does not exhibit external sex differences such as difference in shape or colour. Carassius is omnivorous, spawns several times from May to July. The egg is highly adhesive. Carassius is commonly used for food and is considered by some people more delicious than "Koi" or carp. Moreover, Carassius is a common game fish and large numbers are caught annually. Thus as a material for investigation it is most convenient for the following reasons:

1. It may be captured all the year round.
2. It is abundant in number.
3. It is widely distributed.
4. It is very strong and is easily kept in the aquarium.

Method of Collection and Identification of Sexes.

Fearing that the normal sex ratio cannot be told unless the material represents random sampling, I adopted the method of "Kaibori," or bailing out water, considering that fish caught by hook and line belong to a bigger and more selected group and that nets have two disadvantages,—a large-mesh net allowing the loss of the smaller fish, which pass through it, and a small-mesh net giving the larger fish a chance to escape by means of their greater speed. By the method of "Kaibori", however, we can get all the fish, big and small, which live in a selected area, for the stream is dammed in two places and the water between entirely bailed out. I regret that this method can not be applied to larger streams, owing to the difficulty of bailing water.

To distinguish the sexes by superficial observation is difficult, so I was obliged to dissect all fishes and determine sex directly from the nature of the gonad. The gonad of the male fish is small and is milky white, while that of the female is nearly transparent while immature. Somewhat matured ovaries are light yellow, and wholly matured ones are orange-yellow in colour and granuler in appearance. Thus the sexes can be distinguished very easily after dissection.

The body length was measured by taking the distance between the snout and the tip of the upper lobe of the caudal fin by a divider which reads directly in cm.

THE SEX RATIO IN CARASSIUS LIVING IN A SMALL STREAM.

On September 28, 1924, I investigated the sex ratio of Carassius living in a brook. The particular brook selected is a branch of the Okada canal, which is used for irrigation at Minaminome, Haranomachi town, a suburb of Sendai. The brook is about one and a half meters wide, and one-third meter deep, and is crossed by a small bridge nearly half a meter in width. I stopped with mud, front and rear, a section of the stream under the bridge, two meters in length letting the water of the main stream run into the branches, and then I bailed out water with a bucket. This particular spot appeared to be a faborable place for Carassius, being somewhat shaded by the bridge and a little deeper, while the occasional throwing of garbage here attracts the fish. I collected all fish found here, small and large, the total number of them being 423, of which 281 were from 4 to 6 cm. in length. The result of examination gave the sex ratio of 11.6 males to 100 females (11.6♂ : 100♀). (See Table III, No. 4.)

THE SEX RATIO IN CARASSIUS LIVING IN A SMALL POOL.

I tried the "Kaibori" method at a small water pool behind Yotsumata dam, where there was very small volume of running water. I purposely selected this pool because of the following reasons:

1. The Carassius which lived in this pool were mostly "gold funa" and I had an impression that among "gold funa" the ratio of male fish is comparatively higher.

2. Most of the Carassius living in this pool are very small. Since the sex ratio seems to vary with size the sex ratio of the smaller fishes may be quite different from that of grown fishes.

The area of this pool is about 40 sq. meters and Miscanthus was so luxuriously growing that the land and water were difficult to make out. Its bottom is muddy, with abundant growth of weed, and covered with fallen leaves. The water in the center of the pool was half a meter deep and its outlet was half a meter wide, continuous with a branch of the Okada canal. This pool appeared to be an ideal place for investigation, because the outlet of water was very small and therefore it is possible that Carassius may spawn in this pool, and the hatched embryo remain here for some period before attempting to run into the larger and swifter stream. Altogether 572 Carassius were captured and the majority of them measured from 4 to 6 cm. in length. The result of investigation gave the sex ratio of 18.4♂ : 100♀. (See Table III, No. 8.)

The Sex Ratio in Carassius Living in a Canal.

Thirdly, I wished to investigate the sex ratio of Carassius living in a canal. For this purpose I tried the "Kaibori" method in the Okada canal which runs from the Hirose river, in the same village. In this canal the water voluminous, clear and rapidly running. The width of the canal is 3 to 6 meters and it is one meter or more deep. Finding a comparatively larger number of fishes in a bend of the stream, I bailed out water in this region of 80 square meters, surrounding which a high bank was erected. The Carassius in this canal are rather big, being mostly from 5 to 8 cm. in length. There were several Carassius which measured as long as 20 cm. The total number of Carassius captured were 137, and the sex ratio was 3.8♂ : 100♀. (See Table III, No. 9.)

Normal Sex Ratio.

From the data already given above, as well as from other data I have been gathering in connection with some other observations which were made by the students in our laboratory, the following results were obtained. As will be seen from Table II, the total number of Carassius examined is 1733, and 1535 of them were female and 198 of them were male, this giving the sex ratio of 12.9♂ : 100♀. I believe that this may be taken as the normal sex ratio in Carassius auratus.

TABLE II.

No.	Date	Place	No. of specimen	No. of ♂ :100 ♀
1	Oct. -Dec. 1923	A brook near Masuda Town	285	14.9
2	Dec. 12, „	Gamo Fish-farm near Sendai	12	9.1
3	Sept. 11, 1924	A brook near Iwanuma Town	42	0
4	„ 28, „	A branch of Okada Canal, Haranomati	423	11.6
5	Nov. 14, „	Sendai Fish-market	10	0
6	„ 19, „	A brook near Iwanuma Town	31	0
7	„ „ „	Akanuma, near Sendai	15	0
8	„ 29, „	A pool. Minaminome, Haranomati	572	18.4
9	„ „ „	Okada Canal. Minaminome, Haranomati	137	3.8
10	Apr. -Feb. 1924–1925	Hanuki Canal, near Iwanuma Town	206	12.0
		Total number	1733	12.9

TABLE III.

Body length in cm.	Sex	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Total No.	No. of ♂ :100 ♀
3–4	♀	8			54				28			90	46.6
	♂	4			22				16			42	
4–5	♀	19			156				113	27		315	21.3
	♂	8			16				41	2		67	
5–6	♀	20			105				152	32	7	316	8.9
	♂	6			4				17	1	0	28	
6–7	♀	38		2	46				83	23	31	223	6.7
	♂	3		0	0				5	0	7	15	
7–8	♀	56	1	5	12				38	18	71	201	10.4
	♂	7	0	0	0				4	2	8	21	
8–9	♀	47	2	9	8				20	6	45	132	12.9
	♂	6	0	0	1				5	0	5	17	

9-10	♀	29	4	11	3		4		19	4	22	96	5.2
	♂	2	0	0	0		0		1	0	2	5	
10-11	♀	17	4	7	1		6		19	5	7	66	3.0
	♂	1	1	0	0		0		0	0	0	2	
11-12	♀	10		3		7	13		7	3		43	0
	♂	0		0		0	0		0	0		0	
12-13	♀	3				3	4		2	3		15	0
	♂	0				0	0		0	0		0	
13-14	♀	0		1			3		1	5	1	11	0
	♂			0			0		0	0	0	0	
14-16	♀	1		3			1	1	1	2		9	0
	♂	0		0			0	0	0	0		0	
16-18	♀			1				2		3		6	0
	♂			0				0		0		0	
18-20	♀									1		1	0
	♂									0		0	
20-22	♀							8				8	0
	♂							0				0	
22-25	♀							4				4	0
	♂							0				0	
Total No.		285	12	42	423	10	31	15	572	137	206	1733	

THE SEX RATIO AT BIRTH.

Now let us consider the sex ratio at birth. Pearl (1916) found that in the lake whitefish, Coregonus albus, the sex ratio of adults is the same as that at birth. If the same relation holds in the Carassius we may anticipate that the sex ratio of male to female is equal. Table III shows, however, that the sex ratio in Carassius is 46.6♂ : 100♀ in the young fish of from 3 to 4 cm. in length, while the number of males decreases with the increase of the body length until it reduces to 0♂ : 100♀. From this it is conceivable that the sex ratio of the male at birth may be much higher than the value given above, but I have no direct evidence yet to prove this assumption.

It is curious to find that the sex ratio of the gold fish, which is a variety of Carassius, is 87.2♂ : 100♀ in "riukin," and 100♂ : 100♀ in "wakin." Whether this difference between Carassius and gold-fish in the sex ratio is due to the effect of domestication of the latter can not be definitely stated, though it seems highly probable, and is a very interesting phenomenon for further investigation.

Excess of the Female with the Growth of the Body.

The noticeable thing in Table III is the decrease in the number of males with the increase of the body length, viz. the number of males to each 100 females are:

46.6	3 to 4 cm.	in length
21.3	4 to 5	„
8.9	5 to 6	„
6.7	6 to 7	„
10.4	7 to 8	„
12.9	8 to 9	„
5.2	9 to 10	„
3.0	10 to 11	„
0	11 to 12	„
0	12 to —	„

I was, however, unable to find a male fish which was longer than 11 cm. The anglers tell me also that the bigger fish which are caught with hook and line are mostly females and not males. One might suspect that this abnormal sex ratio is due to the protandric form of reproduction such as Roule, L. reported about Scardinius erythrophthelmus, which belongs to the same family, as Carassius, Cyprinidae. I may say in this connection that I never found one hermaphrodite case, in which one kind of the sex gland was changing to the other kind—that is ovary to testes, or vice versa—out of the 1733 Carassius examined.

Recently Geiser, S. W. (1924) reviewed the literature on the sex ratio of the Top-minnow, Gambusia holbrooki Grd., together with his own observation. He states that in this species the number of females almost invariably surpasses that af the males. Geiser quoted in his paper the observation of Hildebrand (1917) on Gambusia, which reads that "it seems entirely probable that the normal ratio of males to females is about 1 to 8 or 9." We see, then, that there is not a large difference in the sex ratio of Carassius and Gambusia.

The gold-fish is a variety of Carassius produced by domestication for a long period, yet the sex-ratio in gold-fish is approximately 100♂ : 100♀ contrasted with 12.9♂ : 100♀ in Carassius. This is interesting, but difficult to understand. In this connection I may mention that King ('11 & '24) found a slight difference in sex ratio of the wild rat when compared with that of the albino rat, the former giving slightly lower value for the males in spite of the fact that the albino rat is the domesticated variety of Mus norvegicus. On the other hand, in Carassius and gold-fish, in which the latter is the domesticated variety of the former as in the case of the rat, the sex ratio of these two varieties is very different, as was noted above making an interpretation very difficult.

From my own observation, the male of the Carassius or of the goldfish does not eject sperm either when was kept alone, or when large numbers are kept in a small aquarium. The gold fish is generally kept crowded in a small aquarium, or alone in a small dish from a very young stage. Thus the gold fish lacks chance to eject sperm and consequently are as healthy as the females. Carassius on the contrary, lives in the wild state and thus has a good chance to meet females, or in other words, has always a chance to eject sperm. It is conceivable that the Carassius under such circumstance may die from exhaustion as a consequence of excessive excitement. If this assumption is correct we will find a greater death rate in the males than in the females. Furthermore the males most probably mature at the age of 2 years, but the females at the age 3 or 4 (after Prof. T. Higurashi), therefore we will find a greater excess of females over males after the period of reproduction.

However, these explanation applies only after the Carassius reaches sexual maturity, but since the greater preponderance of females over males is already evident before this period, we must still consider other factors for the explanation. As one probable factor, the differential death rate among sexes may be conceivable, and I am carrying out some experiments for testing this hypothesis.

LITERATURE.

1) Geiser, S. W., 1924 Sex-ratio and spermatogenesis in the top-minnow, Gambusia holbrooki Grb. Biol. Bull., Vol. 47.

2) Goldschmidt, R., 1923 The mechanism and physiology of sex determination.

3) Hatai, S., 1918 Metabolic activity of the nervous system, II. The partition of non-protein nitrogen in the brain of the gray snapper (Neomaenis griseus) and also the brain weight in relation to the body length of this fish. Jour. Comp. Neur., Vol. 29.

4) Huxley, J. S., 1923 Late fertilization and sex-ratio in trout. Science, Vol. 58.

5) Kellicott, W. E., 1908 The growth of the brain and viscera in the smooth dogfish (Mustelus canis, Mitchill). Amer. Jour. Anat., Vol. 8.

6) King, H. D., 1911 The sex-ratio in hybrid rats. Biol. Bull., Vol. 21.

7) King, H. D., 1924 Litter production and the sex ratio in various strains of rats. Anat. Rec., Vol. 27.

ON A FORMULA FOR DETERMINING THE WEIGHT OF THE BRAIN OF CARASSIUS AURATUS FROM THE WEIGHT AND LENGTH OF THE ENTIRE BODY, TAKING INTO CONSIDERATION THE REGULAR SEASONAL CHANGES IN THE RELATIVE WEIGHT AND THE DIFFERENCE IN SEX.

BY

KIICHIRO SASAKI.

Donaldson ('02) found a convenient mathematical expression which gives the relation of the weight of the central nervous system to the body weight and body length very satisfactorily. The material chosen by Donaldson for the investigation was the frog, Rana pipiens, and the resulting formula is as follows:

$$\text{Weight C.N.S.} = \log W \times \sqrt{L} \times C$$

Here C.N.S. represents the central nervous system, W the body weight, L the body length, and C the constant. By applying this formula, Donaldson was able to demonstrate the seasonal changes in the weight of the brain of Rana pipiens.

Adopting Donaldson's formula, Komine ('24) recently investigated the central nervous system of the Japanese frog, Rana nigromaculata, and published his observation that the relative weight of the central nervous system changes regularly with the season and according to the sex.

I attempted to investigate the same problem under the direction of Prof. Hatai, and chose Carassius as material for the reason given in the subsequent pages.

I wish to express my hearty thanks to Prof. Hatai, under whose suggestion the present work was undertaken and whose guidance and advice helped me throughout the entire course of the work.

MY OBSERVATION.

On October 13, 1923 I began this investigation and continued until December of the same year. The total number of fish observed was 224, among them 194 female fish and 30 male fish.

The next spring, April 1924, I took up this work again and continued during the next twelve months. The material was always examined in the latter part of every month. In order to obtain uniform results, I observed 12 or more female fish and finished in March 1925. The total number of fish for this period was 187 female and 27 male fish.

MATERIAL USED.

For studying this interesting problem I chose the Funa, or Carassius auratus belonging to the Cyprinidae, Teleostei. The Carassius, as I reported in the previous paper ('25), is widely distributed in Japan and can be captured in every freshwater district.

For the first investigation I collected the material from a brook near Masuda Town, and for the second investigation from the Hanuki canal near Iwanuma Town, all in the neighborhood of Sendai. This particular canal was selected because Carassius are very abundant in it and the water does not freeze in cold weather. In both cases the fish were collected with a hand net.

At the first investigation I wished to observe the relation of the increase in the weight of the brain to the increasing body weight and body length, so I collected Carassius of all sizes, ranging from a total body length of 30 mm. to 145 mm., and a body weight of 0.41 gm. to 38 gms. or more. I got the following formula as the result of this investigation:

$$\text{Brain weight} = \log L \times \sqrt{W} \times C$$

Here L represents the total body length, W the body weight, and C the constant.

At the second investigation I wished to know how the value of the constant changes from month to month, that is, how the relative weight of the brain and its water contents change with the season. With these objects in mind, I collected mature fish, mainly female because they are easy to get, and observed 12 or more fish every month.

TECHNIQUE EMPLOYED.

Carassius is very strong and is easily kept in the laboratory, for example, by renewing the water once a week we can keep Carassius for 5 or 6 months without giving food.

For my first investigation I kept them in a live-box in the pool belonging to the laboratory with a hope to supply many plankton for them. In each case I did not keep them more than one week. For the second investigation, the number which were to be examined each time being smaller, I almost always finished in the first day.

The technique throughout the course of the research was essentially uniform. At first, after scooping up a fish with a small hand net, I wiped the water from its body with a towel, and then took the body weight to the nearest 0.01 gm. Next, the fish was laid on its side and the total body length was determined, taking the distance between the snout and the tip of the upper lobe of the caudal fin by means of a divider which reads directly in mm.

In order to remove the blood from the cranial cavity, I cut off the main blood vessel at the posterior end of the pectoral fin. After that, the brain was exposed by scissors or small bone forceps. In a few instances the coagulated blood in a thin film was found in the cavity but it could easily be taken out with forceps. Then the brain was separated from the spinal cord by an incision between the first vertebra and the base of the skull, the olfactory nerves were cut close to the cerebrum, and the pituitary body was removed. The brain was then placed in a small bottle, which had been previously weighed and preserved in a desiccator.

The gonad was weighed and used for correcting the body weight.

The fresh weight of the brain was determined by weighing the bottle again to a milligram, then the bottle with its contents was placed in a steam oven at a temperature at 90° to 95°C. for one week, and weighed. The difference between the fresh weight and the dry weight gives the weight of the water.

I. THE RELATION BETWEEN THE BODY WEIGHT AND THE TOTAL BODY LENGTH.

The relation between the body weight and the total body length gives the following ratios. The ratio obtained by dividing the body length by the body weight diminishes as the body weight increases. (See Table 1 and 2.)

TABLE 1.

Data on Female Fish.

No. of specimen	Body weight gms.	Body length mm.	Ratios
14	0.72	39.7	55.1
27	1.47	50.7	34.2
16	2.64	60.3	22.8
18	3.51	65.6	18.7
15	4.61	70.0	15.2
12	5.62	75.9	13.5
16	6.54	78.4	12.0
13	7.56	82.1	10.9
10	8.51	85.5	10.0
5	9.53	87.8	9.2
8	10.64	91.8	8.6
5	11.43	93.2	8.4
7	12.32	95.0	7.7
13	15.17	102.8	6.8
5	19.06	109.0	5.7
5	22.14	113.0	5.1
4	27.04	120.8	4.5
1	38.23	145.0	3.8

TABLE 2.

Data on Male Fish.

No. of specimen	Body weight gms.	Body length mm.	Ratios
6	0.74	40.0	54.1
9	1.39	48.3	34.7
4	2.62	58.8	22.4
1	3.48	63.0	18.1
2	4.68	70.5	15.1

2	5.64	73.0	12.9
1	6.53	77.0	11.8
1	8.45	85.0	10.1
1	9.50	85.0	9.0
3	10.67	88.0	8.3

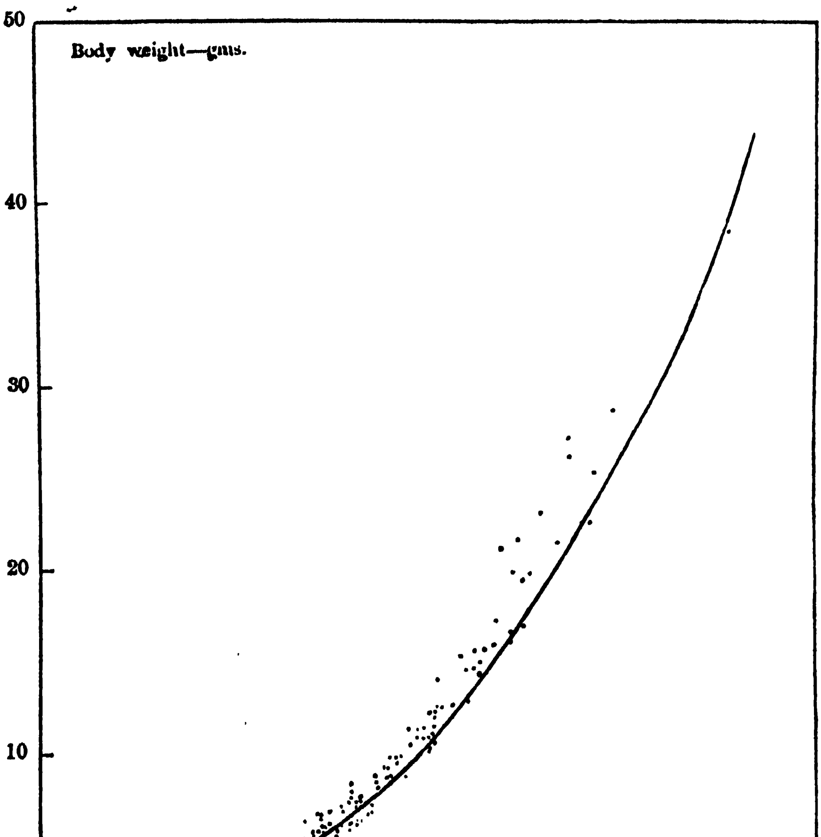


CHART I. Showing relation of weight to length in 194 female fish.

From Table 1 and 2 we can see that among Carassius of the same body weight the females have a slightly greater body length than the males. But by Hecht on the dog fish ('13) and weakfish ('14), and by Heincke on the European plaice ('07) similar sex difference was not observed.

Donaldson ('09) observed such a phenomenon in the albino rat, and reported that the ratio diminishes as the body weight increases, and also that there is a slight difference in the ratio between the sexes. But in the albino rat, the females have a slightly shorter body length for a given body weight than the males.

It is reported by Hecht, Crozier, and others that there is the following mathematical relation in the fish between the body length and the body weight:

$$Y = a\ X^3$$

In which Y represents the body weight, X the body length, and a the constant.

In Carassius, according to my present data, if the body weight is expressed in gms., and the length in cms., the following relation is given:

$$\text{Body weight in gms.} = 0.0131\ (\text{Body length in cm.})^3$$

How the constant, 0.0131, which was found in Carassius, stands among the values found in other fish I gathered together in Table 3 according to data given by Hecht ('16) and others.

TABLE 3.

Weight-length constant.

Species	$a \times 10^2$	Observer
Mustelus canis	0.274	Hecht ('16)
Brevoortia tyrannus	0.912	"
Clupea sprattus	0.695	Jenkins ('02)
" harengus	0.631	"
Anchovia brownii	0.709	Hecht ('16)
" mitchilli	0.618	"
Peprilus alepidotus	1.70	"
Cynoscion regalis	0.877	Crozier & Hecht ('14)
Leiostomus xanthurus	1.15	Hecht ('16)
Orthopristis chrysopterus	1.30	"

Pleuronectes playessa	1.07	Johnstone ('12)
Carassius auratus	1.31	Sasaki

The value of this constant, however, can not be absolute, because the weight-length relation may alter considerably according to the physiological changes that the species undergoes in the matter of nutrition, reproduction, and hibernation. Furthermore, there will be a change due to the locality and climate. I am in full agreement with the opinion of Johnstone ('11), who states about the plaice that "this coefficient is only convenient index of the 'condition' of plaice inhabiting any given fishing ground."

Making use of the data which I got at the second investigation, I will show the changes of the constant from month to month. (See Table 4.)

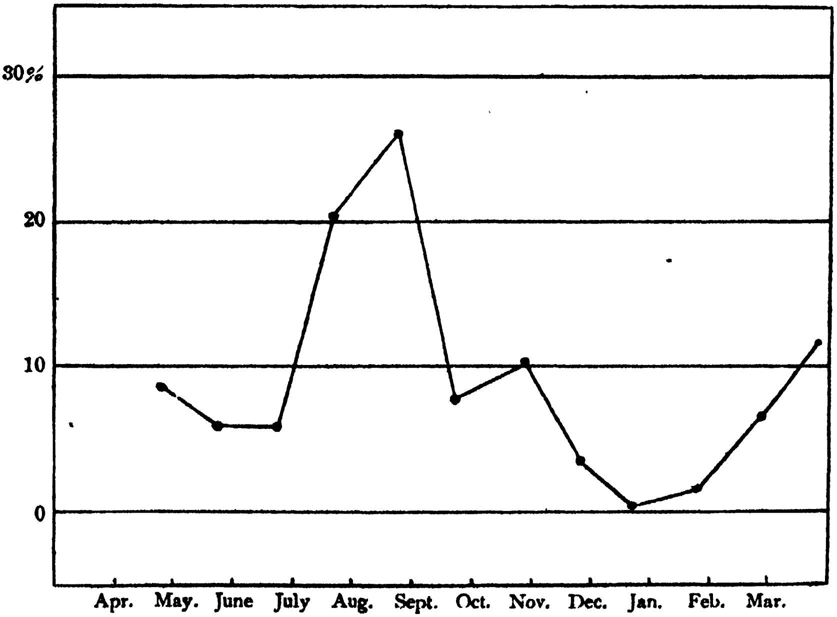


CHART II. Periodic annual change in the weight-length ratio in the female fish.

TABLE 4.

Data on Female Fish.

Month	Body length obs. cm.	Body weight		
		obs. gms.	cal. gms.	dif./cal. × 100
Apr. ('24)	8.37	8.34	7.68	8.59
May	9.57	12.17	11.48	6.01
June	7.82	6.63	6.26	5.91
July	7.84	7.59	6.31	20.29
Aug.	7.28	6.38	5.05	26.34
Sept.	7.66	6.33	5.89	7.47
Oct.	7.34	5.71	5.18	10.23
Nov.	8.03	7.03	6.78	3.69
Dec.	7.85	6.37	6.34	0.47
Jan. ('25)	7.19	4.96	4.87	1.85
Feb.	7.54	5.98	5.62	6.41
Mar.	7.55	6.31	5.64	11.88

As is shown Table 4, the weight of the body in relation to the body length is much lighter in the months of December, January, and February, owing to the state of hibernation, but in March the fish become active and take food voraciously, thus becoming heavier again. However, in May and June the body weight again decreases owing probably, as in the case of salmon (Miescher '89, '97, and others), to wasting of the muscle and other tissues for the production of sperm and ova, as well as to fatigue as a consequence of sexual excitement. In July and August, immediately after the reproductive period, the fish take food intensively and the body increases in weight considerably and gives maximum value in weight. After August the fish gradually become thinner till the period of hibernation, which begins in December. This gradual falling off in weight may be due to lowering temperature and consequently to lesser appetite.

The change of the weight-length relation with the season in the plaice is given by Thompson in his work on "Growth and Form" and I have cited the entire table for the purpose of comparison with my own observations on Carassius.

TABLE 5.

Relation of weight to length from month to month in plaice of 55 cm. in length. (Cited from "Growth and Form" Thompson 1917 pp. 101.)

Month	Average weight in gms.	$W/L^3 \times 100$	W/L^3 smoothed
Jan.	2039	1.226	1.157
Feb.	1735	1.043	1.080
Mar.	1616	0.971	0.989
Apr.	1585	0.953	0.967
May	1624	0.976	0.985
June	1707	1.026	1.005
July	1686	1.013	1.037
Aug.	1783	1.072	1.042
Sept.	1738	1.042	1.111
Oct.	2029	1.220	1.160
Nov.	2026	1.218	1.213
Dec.	1998	1.201	1.215

Thompson explains "with unchanging length, the weight and therefore the bulk of the fish falls off from about November to March or April, and again between May or June and November the bulk and weight gradually restored. The explanation is simple, and depends wholly on the process of spawning, and on the subsequent building up again of the tissue and the reproductive organs."

Comparing the seasonal changes in plaice and Carassius, it will be recognized that there is a time in which the plaice falls off in weight because of the process of reproduction, but in the Carassius there are two such periods, one due to the process of reproduction and the other to hibernation. (See Table 4 and Chart II.)

II. ON THE RELATION BETWEEN THE BRAIN WEIGHT AND THE TOTAL BODY LENGTH AND WEIGHT.

Making use of the data obtained at the first investigation, I wished to get a relation between the brain weight and the body weight. I have attempted to apply the formula which was suggested by Donaldson, that is, C. N. S.$=\log W \times \sqrt[4]{L} \times C$. It was soon found that the value obtained by multiplying the logarithm of the body weight by some root of the length of the body was not suitable in this case. Thinking that the form of Carassius differs from the frog, I tried to multiply the logarithm of the body length by some root of the body weight. After trials, I found that this modification of the original formula of Donaldson gives more satisfactory results, that is,

$$\text{Brain weight} = \log L \times \sqrt{W} \times C$$

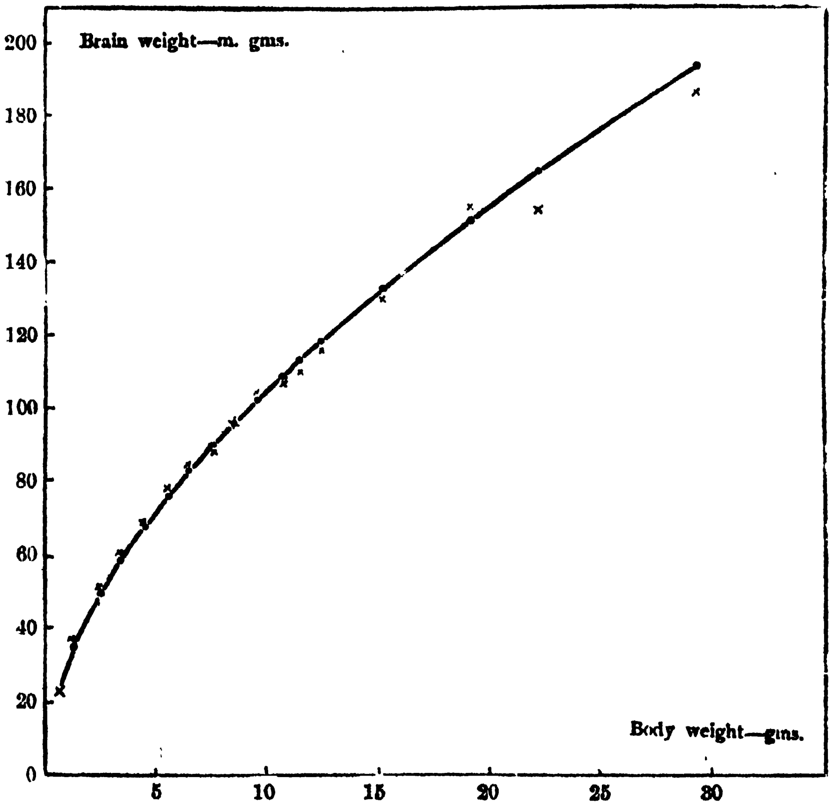


CHART. III. Showing the weight of the brain in female fish in relation to body weight.

× Observed • Calculated

Here the weight of brain, in milligrams, is made equal to the logarithm of L, the body length, expressed in millimeters, multiplied by the square root of W, the weight of the body, in grams, the product of these factors being raised to the value of the observed weight of the brain by multiplying by a constant C. This constant, in the present case, has the value of 17.1, fluctuating from 15.9 to 17.9.

This formula differs from Donaldson's formula for the frog in taking the logarithm of the body length instead of the body weight. This modification was necessary because of the difference in the morphology of fish from frogs. Furthermore, I have used only the brain weight, while Donaldson used the entire central nervous system in his formula.

In Table 6 are given the values of the observed brain weight contrasted with the calculated weight of brain according to the formula given above.

TABLE 6.

Data on Female Fish.

Weight range	No. of specimen	Mean body weight gms.	Mean total body length mm.	Value of constant	Mean brain weight mgms.	
					obs.	cal.
0-1	14	0.72	39.7	17.1	23.2	23.2
1-2	27	1.47	50.3	17.9	36.9	35.3
2-3	16	2.64	60.3	17.5	50.6	49.5
3-4	18	3.51	65.6	17.5	59.6	58.2
4-5	15	4.61	70.0	17.4	69.0	67.7
5-6	12	5.62	75.9	17.6	78.3	76.2
6-7	16	6.54	78.4	17.6	84.8	82.8
7-8	13	7.56	82.1	16.8	88.3	89.9
8-9	10	8.51	85.5	17.1	96.5	96.5
9-10	5	9.53	87.8	17.5	105.2	102.6
10-11	8.	10.64	'91.8	16.8	107.8	109.4
11-12	5	11.43	93.2	16.6	110.4	113.9
12-13	7	12.32	95.0	16.7	116.2	118.7
13-17	13	15.17	102.8	16.6	130.0	134.0
17-21	5	19.06	109.0	17.6	156.2	152.1
21-25	5	22.14	113.0	16.0	154.5	165.2
25-38	5	29.28	125.6	16.4	186.9	194.2

Concerning the same relation, Kellicott ('08) studied the smooth dog-fish and plotting the body weight on the base line and the brain weight on the ordinate, he drew an ideal curve by hand. But he does not record the body length in his paper so I regret that I could not test the applicability of my formula to it. Hatai ('18) investigated about 74 gray-snappers and found that the relation of the brain weight to the increasing body length is practically linear and may be satisfactorily expressed as $Y=a \times bX$, in which Y represents brain weight, X length, and a and b are the constants. In the albino rat, Donaldson ('09) found that the relation between the brain weight and body weight or body length can be represented by a logarithmic curve.

From the formula, Brain weight $= \log L \times \sqrt{W} \times C$, we can at once determine the value of C from the relation:

$$C=\frac{\text{Brain weight}}{\log L \times \sqrt{W}}.$$

We find then the value of C varies directly with the weight of the brain. It becomes, therefore, possible to express the changes in the relative weight of the brain by the difference in the value of C quite independently of the absolute body size of Carassius.

By this method, comparing the value of the constant, I wished to know if the value of constant changes from month to month and the results are given in Tables 7 and 8.

TABLE 7.

Data on Female Fish.

Date	No. of specimen	Mean body weight gms.	Mean body length mm.	Mean brain weight mgms.	Value of constant
Apr. 26, 1924	18	8.34	83.7	95.4	17.2
May 23	18	12.17	95.7	114.3	16.5
June 26	11	6.43	78.2	81.5	16.7
July 19	15	7.59	78.4	81.2	15.6
Aug. 26	14	6.38	72.8	78.0	16.6
Sept 25	20	6.33	76.6	74.5	15.7
Oct. 27	13	5.71	73.4	76.1	17.1
Nov. 26	19	7.03	80.3	98.6	19.5
Dec. 20	15	6.37	78.5	93.8	19.6

Jan. 27, 1925	16	4.96	71.9	77.6	18.7
Feb. 26	20	5.98	75.4	82.2	17.9
Mar. 26	13	6.31	75.5	78.5	16.6

TABLE 8.

Data on Male Fish.

Date	No. of specimen	Mean body weight gms.	Mean body length mgms.	Mean brain weight mgms	Value of constant
Apr. 26, 1924	2	7.99	82	96.1	17.8
May 23	3	4.20	69	67.1	17.8
June 26	5	6.47	76	90.6	18.9
July 19	2	7.75	79	89.6	17.0
Aug. 26	0	—	—	—	—
Sept. 25	0	—	..	—	—
Oct. 27	5	5.66	74	79.2	17.8
Nov. 26	1	8.57	83	106.7	19.0
Dec. 20	1	6.30	77	87.1	18.4
Jan. 27, 1925	1	7.35	81	94.1	18.2
Feb. 26	2	4.17	67	67.5	18.1
Mar. 26	5	6.85	78	86.7	17.9

The value of the constant, that is, the relative weight of the brain, is low in spring, after hibernation, and in summer, after the process of reproduction, but is high in winter before hibernation.

In other words, in March, after the hibernation, the relative weight of the brain is low and becomes higher from the beginning of April. In May or June the fish reproduce sperm and ova and some of them continue the process until August, according to the environment. In my present data, the constant in July is minimum for the year round, and in August there is slight recovery, in September it falls a little again, perhaps resulting from the postponement of the process of reproduction in some of the specimen. October and November are months in which the fish prepares for hibernation and then also the value of constant reaches maximum. From the middle of December to March of the next year, the fish hibernate and the value of constant becomes lower and lower.

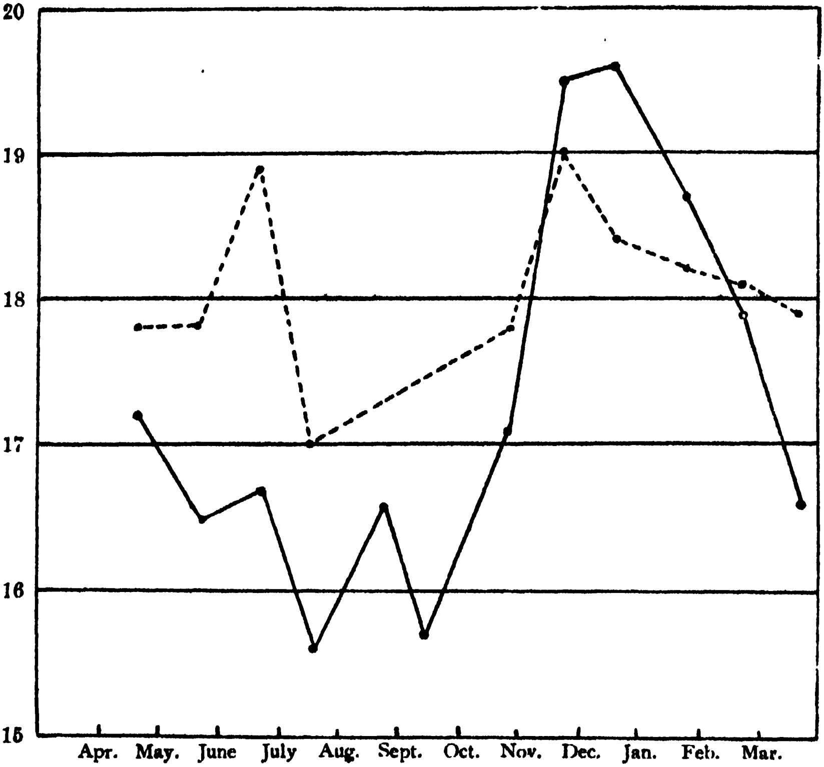


CHART IV. Showing the value of the constant of the brain according to the season.
—— Female fish ········ Male fish

Such an increase and decrease of the value of the constant (the relative weight of brain), corresponds with nutrition, reproduction, and hibernation, as the body length and weight do (see page 246). It is said generally that in the higher animals, for example in Mammalia, the weight of the brain is less changeable under varied physiological states, for instance, Marchand ('02) in men, and Findley ('21) in pigeons and fowls, report that there is no loss in the brain weight as the result of inanition. On the other hand, Porter ('85—'87, '89) published results found by autopsies upon victims of the Madras famine in which he reports that in extreme cases there was an apparent loss of about

9.8% of the brain weight in the men and 8.1% in the women, while all oft hese had probably lost over 40% in body weight. He concluded, therefore, that the brain wastes like other tissue, though not so severely.

According to Matiegka ('04) the brain is affected by the nutritional condition. Comparing extremes of good and poor condition in adults from 20 to 59 years of age, the brain weight under the former condition was heavier than the later to the extent of about 7.3% for the men and of 5.6% for the women. Valentin ('57) noted weights indicating a loss of about 5% in the brain of marmots with a loss of 35.5% in body weight after 166 days of hibernation, so we can see that there are reductions in the brain weight due to the lack of nutrition and to hibernation.

It is conceivable from the structure of the brain, that in lower vertebrates, such as fish, it may undergo still greater modification under the same unfavourable condition than in the higher vertebrates, especially the higher mammals.

Seasonal changes of the value of the constant in the frog were published by Donaldson ('11) and by Komine ('24). They say "the relative weight of the central nervous system is low at the time of emergence, high in the midsummer (July) and low again at the time of hibernation. During hibernation it remains nearly constant".

Komine ('24) compared the value of the constant in Rana nigromaculata with R. pipiens, R. esculenta, and R. temporaria, and said that these differences may partly be due to the difference of climate, location, and food.

However, Donaldson interpretes the seasonal change in the relative weight of the central nervous system in the following words, "This variation in the relative weight according to season is due to lack of coincidence between the growth of the central nervous system and the growth of the entire body", and Komine found, besides the changes of the brain weight, the corresponding changes of the water contents, as well as the deposition of non-protein substance.

In the case of Carassius we also notice lack of coincidence between the growth of brain and the growth of the entire body, as was suggested by Donaldson. I make this statement from the fact that the variation of the constants in the formula $Br. = \log L \times \sqrt{W} \times C$ is nearly opposite the variation of constants given by the formula $Y = aX^a$. Such relation is shown in Charts II and IV. From this we can safely conclude that the body does not grow throughout the entire season in uniform relation with the brain. What the responsible factor is in producing such non-uniformity of growth between brain and body can not be definitely stated but we may mention the following as probable causes: first, inanition during hibernation and reproduction, second, production of gonads and exhaustion in the process of reproduction, both of which may waste the tissue, including the brain.

Some will doubt that Carassius fasts during the process of reproduction but it is a well known fact among anglers that they can not hook Carassius by bait at the time of reproduction and during hibernation. But it is very easy in the spring (April) in the emergence from the hibernation, or after reproduction.

III. ON THE WATER CONTENTS OF THE BRAIN.

The percentage of water shows very slight variation in the small fish of 0.4 gms to the large fish of 38 gms., tending to diminish as the body weight increases. (See Table 9.)

TABLE 9.

Data on Female Fish.

Weight range gms.	No. of specimen	Mean water contents	Mean brain weight mgms.
0-1	14	81.30%	23.2
1-2	27	81.64	36.9
2-3	16	81.76	50.6
3-4	18	81.88	59.6
4-5	15	82.69	69.0
5-6	12	81.66	78.3
6-7	16	82.09	84.8
7-8	13	81.99	88.3
8-9	10	81.15	96.5
9-10	5	81.28	105.2
10-11	8	80.81	107.8
11-12	5	81.32	110.4
12-13	7	80.60	116.2

13–17	13	81.00	130.0
17–21	5	81.10	156.2
21–25	5	80.42	154.5
25–38	5	80.80	186.9

Table 10.

Data on Male Fish.

Weight range gms.	No. of specimen	Mean water contents	Mean brain weight mgms.
0–1	6	80.82%	28.2
1–2	9	81.03	32.8
2–3	4	81.01	47.3
3–4	0	—	—
4–5	0	—	—
5–6	2	79.73	80.0
6-7	1	80.11	75.4
7–8	0	—	—
8–9	1	81.43	112.0
9–10	1	80.40	100.0
10–11	3	80.63	103.9

Similar relations were observed by Donaldson ('05) in the summer flounder, by Scott ('12) in the smooth dog-fish, and by Hatai ('18) in the brain of the gray-snapper.

The greater amount of water is found, as a rule, in the younger system than in the older. It is a fact, proved by Donaldson ('16) and others, that in the central nervous system the water content decreases with the growth of the organ. We know by Donaldson's report ('10) that in the albino rat the water content of the brain at birth is 87.8%, which diminishes in maturity to 77.5%. During the first 25 days there is a rapid decrease of 6%, that is during the time of greatest growth of the central nervous system, and Donaldson ('16) reports that the water contents in the brain correspond to the deposits of the myelin substance, that is to say, the water contents diminish with the increase of myelin.

Now in Carassius, according to my present data, there is no large difference in the amount of water contained in the brain, but there is a slow decrease as the body weight increases, so it may be supposed that the brain develops during the 2 or 3 months after the fish are hatched and that the process of myelination occurs rapidly, but during the subsequent long period it must be very slight.

We know that there is a slight difference in the water contents of the brain according to sex, that is, the male fish contains less, but it is not peculiar to Carassius as there are reports by Hatai ('18) of the gray-snapper giving 77.4% for the male fish and 78.1% for the female and by Komine ('24) of the frog, Rana nigromaculata (♀ 84.5%, ♂ 84.0%). Table 11 gives data published in recent years concerning the water contents of the brain of fishes.

TABLE 11.

Species	Water contents in the brain	Authority
Summer flounder	78.4%	Donaldson ('05)
Smooth dog-fish	78.5	Scott ('12)
Gray-snapper	78.1(♀), 77.4(♂)	Hatai ('18)
Gold fish	82.2	Sasaki (Data not yet published)
Carassius auratus	81.46(♀), 80.79(♂)	,, ('23)
,,	82.15(♀), 81.34(♂)	,, ('24-'25)

As will be seen from Table 11, the brain of Carassius contains 2 or 3% more water than the others. In my investigation on the water contents of gold fish 82.2% is shown, which is also higher by 3% or more than the data given by Donaldson, Scott, and Hatai. As far as the limited data given above are concerned, the marine fish contain less water in the brain than the fresh water fish, though I can not give the reason why these two forms differ from one another.

One can see from Table 12 the percentage of the water changes from month to month, August giving maximum and April minimum.

Furthermore, the water percentage is minimum in April and then increases little by little until in August it shows maximum. In September and October it diminishes a little but in November increases again. Then the fish hibernate until the end of March and the water contents also diminishes slowly.

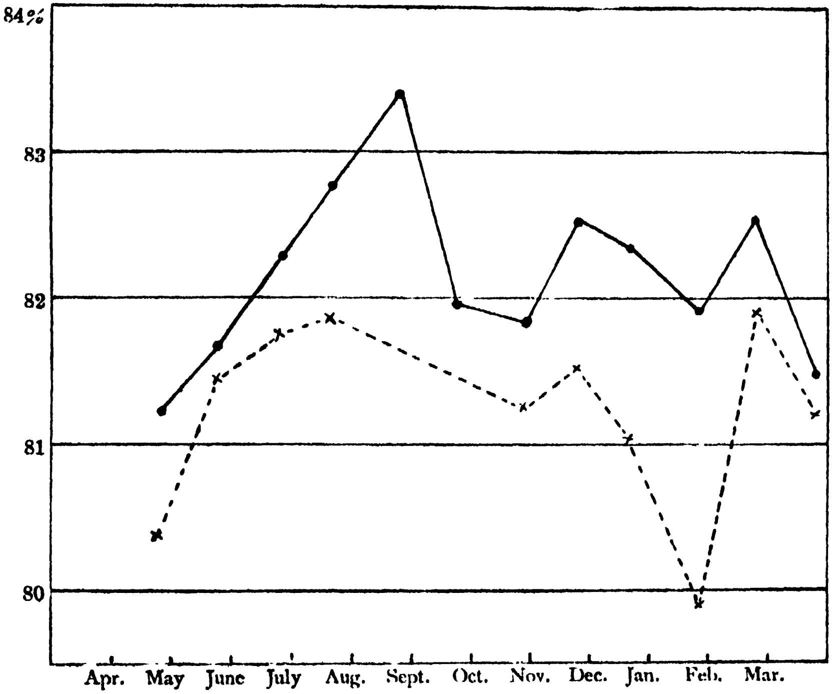


CHART V. Showing the water percentage according to season.

—•— Female ---x--- Male

TABLE 12.

Month	Female		Male	
	No. of specimen	Water contents	No. of specimen	Water contents
Apr. 26 ('24)	18	81.22%	2	80.37%
May 23	13	81.66	3	81.46
June 26	11	82.27	5	81.74
July 19	15	82.75	2	81.86
Aug. 26	14	83.41	0	—
Sept. 25	20	81.97	0	—
Oct. 27	13	81.85	5	81.26

Nov. 26	19	82.55	1	81.54
Dec. 20	15	82.36	1	81.06
Jan. 27 ('25)	16	81.92	1	79.92
Feb. 26	20	82.57	2	81.92
Mar. 26	13	81.49	5	81.21

Donaldson ('11) and Komine ('24) published data on the water content in the frog. Komine reported that "the water content of the central nervous system of Rana nigromaculata increases in the summer and decreases in the spring. This can be seen in the Table of Donaldson's study on the seasonal changes in the relative weight of the central nervous system of the leopard frog." He did not explain by what reason the water content of the brain changes by the season but reports that the water content and the amount of the non-protein nitrogen stands in reverse proportion.

On the other hand, reading the same paper of Donaldson ('11) which was cited by Komine, we can see "it is important to state that in both the brain and spinal cord no systematic variation in the percentage of water can be observed during active season." But on the percentage of water in the entire body he reports as follows, "During the active season, the percentage of water in the entire frog falls slightly from spring to summer and rises from summer to autumn. These changes seem to be due to the combined effects of advancing age and varying food supply."

From Donaldson's and Komine's reports in the frog we notice there is a slight difference of about 1% in the percentage of water of the brain by season but in Carassius we can see that there is a difference of 2.2% when the maxima and minima are contrasted. I do not know how the water content is related to the non-protein nitrogen in Carassius, but I can say from the data that the water content is high in the season when the metabolism is most active and at the time when the brain is heaviest.

SUMMARY.

1. In Carassius, the ratios of the body weight and the total body length diminish as the total body length increases.

2. Among Carassius of the same body weight, the females have a slightly greater body length than the males.

3. The relation between the body length and the body weight is shown by the formula,

$$\text{Body weight in gms.} = 0.0131\ (\text{Body length in cm.})^3.$$

4. The value of constant changes regularly with the season. Generally speaking, within the whole year, in July and August it is highest, in December lowest.

5. Such a regular change of the constant of the weight-length relation may be mainly due to lack of nutrition during hibernation, and to reproduction and exhaustion.

6. The relation between the brain weight, the body weight, and total body length is shown by the formula:

$$\text{Brain weight in mgms.} = \log\ (\text{Total body length in mm.}) \times \sqrt{\text{Body weight in gms.}} \times 17.1$$

7. The relative weight of the brain or the value of the constant changes in the fish, as Donaldson found in the frog, with the season, and in Carassius we find that the value of the constant is low in spring, after hibernation, in summer, after the process of reproduction, and high in winter, before going into hibernation.

8. Such an increase and decrease in the relative weight of the brain may be due to two reasons:

1. Inanition during hibernation and reproduction.

2. Production of the gonad and exhaustion in the process of reproduction, which wastes the tissues, and perhaps also the brain.

9. The water contents of the brain is slightly greater in females than in males. (See Table 12.)

10. The water contents changes also with the season. It reaches maximum in August and minimum in April, that is, the water contents is high in the season the metabolism is most active and when the brain is heaviest, and is low under reverse conditions.

LITERATURE.

1) CROZIER, W. J. and HECHT, S., Correlations of weight, length, and other body measurement in the weakfish, Cynoscion regalis. (Bull. Bur. Fish., Vol. 33, pp. 141. 1913.)

2) DONALDSON, H. H., On a formula for determining the weight of the central nervous system of the frog from the weight and length of its entire body. (The Univ. Chicago Press 1902.)

3) DONALDSON, H. H., On the percentage of water in the brain and in the spinal cord of the albino rat. (Jour. Comp. Neur., Vol. 20, 1910.)

4) DONALDSON, H. H., On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog. (Jour. Morph., Vol. 22, 1911.)

5) DONALDSON, H. H., A preliminary determination of the part played by myelin in reducing the water content of the mammalian nervous system (albino rat). (Jour. Comp. Neur., Vol. 26, 1916.)

6) DONALDSON, H. H., On the relation of the body length to the body weight and to the weight of the brain and of the spinal cord in the albino rat (Mus norvegicus var. albus). (Jour. Comp. Neur., Vol. 19, 1919.)

7) HATAI, S., Metabolic activity of the nervous system. II. The partition of non-protein nitrogen in the brain of the gray-snapper (Neomaenis griseus) and also the brain weight in relation to the body length of this fish. (Jour. Comp. Neur., Vol. 29, 1918.)

8) HATAI, S., The brain weight in relation to the body length and also the partition of non-protein nitrogen in the brain of the gray-snapper (Neomaenis griseus). (Proc. Nat. Acad. Sci., Vol. 4, 1918)

9) HECHT, S., Form and growth in fishes. (Jour. Morph., Vol. 27, 1916.)

10) JACKSON, C. M., The effects of inanition and malnutrition upon growth and structure. 1925.

11) KELLICOTT, W. E., The growth of the brain and vicera in the smooth dog-fish (Mustelus canis Mitchill). (Amer. Jour. Anat., Vol. 8, 1908.)

12) KOMINE, S., Metabolic activities of the nervous system. V. 3. On the regular seasonal changes in the relative weight and the sex difference of the central nervous system of Rana nigromaculata (Sci. Report Tohoku Imp. Univ. 4th series. Vol. 1, 1924.)

13) SASAKI, K., On the sex ratio in Carassius auratus. (Sci. Report Tohoku Imp. Univ. 4th series, Vol. 1, 1925.)

14) THOMPSON, D. W., On growth and form. 1917.

WEITERE UNTERSUCHUNGEN ÜBER DIE LEBENSDAUER DER WEIDENSAMEN.

VON

YÔZÔ NAKAJIMA.

Dass die Lebensdauer der Samen je nach der Art sehr ungleich ist, ist seit langem bekannt. Es gibt Samen, welche noch nach einigen Jahrzehnten keimfähig sind, wie De Candolle, Becquerel, Nobbe und andere gezeigt haben. Diese langlebigen gehören meistens den harten Samen der Leguminosen, Malvaceen u. a. an. Neben diesen sehr langlebigen gibt es auch sehr kurzlebige. Nach Hildebrand werden die Samen von Oxalis rubella und deren Verwandte durch Austrocknen leicht getötet. Auch ist bekannt, dass manche Wasserpflanzensamen gegen Austrocknen sehr empfindlich sind. Nach meinen Erfahrungen verlieren die Samen von Trapa natans ihre Keimfähigkeit schon nach einer nur wenige Tage andauernden Austrocknung unter dem direkten Sonnenlicht. Wie ich schon früher (Nakajima 1919) zeigte, werden auch die Samen von Aucuba japonica, Fatsia japonica, Mahonia japonica, Liriope graminifolia, var. communis, Gymostemma pedatum durch Austrocknen geschädigt, und dasselbe gilt nach meinen Erfahrungen auch von Podocarpus macrophylla, subsp. Maki, Eriobotrya japonica und Ginkgo biloba. Die Lebensdauer aller dieser Samen kann man nur durch Verhinderung des Austrocknens auffallend verlängern.

Seit langem weiss man, dass die Samen der Salicaceen nur eine sehr kurze Keimfähigkeitsdauer haben. So erwähnt Wichura (1865) gelegentlich seiner Bastardexperimente mit verschiedenen Weiden, dass die Weidensamen nur fünf oder sechs Tage keimfähig bleiben. Nobbe (1876) spricht in seiner „Samenkunde" über die Kurzlebigkeit des Embryos der Ulme, Pappel und Weide. Schröder (1886) hat mit Salix fragilis und Populus nigra Versuche gemacht und bemerkt, dass Salix nach 25 Tagen, gleichviel ob frei an der Luft liegend oder aber in einer kleinen Glasröhre durch dichten Verschluss geschützt, gleichviel ob im Licht oder im Dunkeln aufbewahrt, die Keimkraft einbüsst.

Und Pappelsamen büsste, während 13 Tagen im Exsikkator gelegen, seine Keimkraft zur Hälfte, nach 30 Tagen, lufttrocken oder in einer geschlossenen Glasröhre aufbewahrt, seine ganze ein. In seiner „Biologie der Pflanzen" gibt Wiesner (1889) an, dass Salix purpurea nach 41 Tagen zu 21%, nach 60 Tagen zu 10% und nach 85 Tagen zu 8% Keimung zeigt. Populus nigra war noch kurzlebiger und verlor nach 50 Tagen seine Keimkraft völlig. Er hat auch bemerkt, dass, wenn Salix bei 70° und Populus bei 60° getrocknet werden, sie an Keimkraft nicht leiden. Bei Salix pentandra konnte Woloszezak (1889) eine Lebensdauer von 48 Tagen feststellen. Leider konnte er seine Untersuchungen nicht weiter fortsetzen, da er keine Samen mehr vorrätig hatte. Mit Rücksicht auf die Angabe von Wiesner fügt er hinzu: „Mir scheint es jedoch, dass die Zeit von 85 Tagen (das zeigt Wiesners Resultat) keine aüsserste Grenze für die Keimungsfähigkeitsdauer der Weidensamen überhaupt bilden." Shirasawa und Inamura (1906) geben an, dass die Samen von Populus tremula var. villosa nur eine Keimfähigkeitsdauer von 30–42 Tagen haben. Oyama (1911) hat mit denselben Samen experimentiert und sie in verschiedener Weise aufbewahrt. Die längste Keimfähigkeitsdauer beobachtete er, wenn er die Samen ½–1 Stunde im Tageslichte trocknete, dann im Glase dicht verschloss und im Keller aufbewahrte. Dann keimten sie noch nach etwa 70 Tagen. Wurden die Samen aber im offenen Glase belassen, zeigten sie nur eine Lebensdauer von 17–21 Tagen. Nohara (1913) hat mit den Samen von Salix rubra und S. rubra × S. purpurea subsp. amplexicaulis folgende drei Aufbewahrungsversuche gemacht. 1. im Exsikkator über Chlorcalcium, 2. im Zimmer an der Luft, 3. in mit Wasserdampf gesättigtem Raum. Das Resultat war folgendes. Bei 1) war die Keimkraftdauer nach 1–3 Tagen, bei 2) nach 12–15 Tagen und bei 3) nach 7–12 Tagen erloschen. Kinzel (1915 u. 1920) untersuchte verschiedene Weiden und bemerkte, dass die Lebensdauer der Weidensamen sehr kurz ist, aber nach der Art verschieden, und dass die Samen der Alpen- oder arktischen Weiden beträchtlich widerstandfähiger gegen Austrocknen sind als die der in der Ebene wachsenden Weidenarten. Oyama (1920) hat mit denselben Objekten wie früher noch weitere Versuche ausgeführt, und diesmal ist es ihm gelungen, ihre Lebensdauer bis zu 165 Tagen zu verlängern dadurch, dass er die Samen zuerst mit Chlorcalcium während 30

Tagen trocknete und dann im Glasgefäss dicht verschlossen aufbewahrte.

Wie aus der Literatur-Übersicht zu ersehen ist, liegen bereits viele Versuche über die Lebensdauer der Weidensamen vor, aber es scheint mir, dass der Einfluss der Luftfeuchtigkeit noch zu wenig in Betracht gezogen wurde. Wie wichtig z. B. dieser Einfluss für die Lebensdauer der Pollenkörner ist, geht aus der Arbeit von Pfundt hervor. Ich begann daher schon in meiner letzten Abhandlung (Nakajima 1921) den Einfluss der Luftfeuchtigkeit auf die Keimfähigkeitsdauer der Weidensamen zu studieren. Um verschiedene Luftfeuchtigkeit zu bekommen, wendete ich Schwefelsäure, Chlorcalcium, Ätzkali, gebrannten Kalk und Wassermischungen dieser Stoffe an, ferner benutzte ich auch Mittel wie Glyzerin u. s. w. Die Weidenarten, welche ich bei meinen Versuchen hauptsächlich verwendete, waren Salix pierotii und S. japonica. Alle diese Samen büssten ihre Keimkraft schon nach einer Woche ein, wenn man sie im Zimmer an der Luft oder im Glas dicht verschlossen liegen liess. Desgleichen, wenn man sie über Schwefelsäure, Chlorcalcium und anderen stark hygroskopischen Mitteln aufbewahrte. Wenn man aber diesen hygroskopischen Mitteln eine mässige Wassermenge zusetzte, z. B. zu konzentrierter Schwefelsäure ein gleiches Volumen Wasser und etwa 15% Dampfspannung gewann und dann über dieser Mischung die Samen aufbewahrte, so wurde die Lebensdauer zu meiner Überraschung ausserordentlich stark verlängert, auch bei Anwendung von Mischungen von Chlorcalcium, Ätzkalk und anderen. Auch Glyzerin, ein mässiges Trockenmittel, gab gute Resultate. Ich zeigte auch, dass die Weidensamen, selbst wenn sie in für die Erhaltung der Keimfähigkeit günstiger Luftfeuchtigkeit aufbewahrt wurden, schliesslich doch zu Grunde gehen, wobei die hohe Temperatur des Sommers beschleunigend wirken dürfte. Als ich eine Probe, welche über gemischtem Chlorcalcium in einem kleinen Glasgefäss aufbewahrt war, in einem kühlen Brunnen von Mai bis Oktober versenkt liess, dann herausnahm und im Laboratorium von Zeit zu Zeit auf ihre Keimkraft prüfte, konnte ich noch nach 320 Tagen 2% gute Keimung bekommen. Im zweiten Jahre untersuchte ich ausser den oben erwähnten zwei Arten auch die alpine Weide, Salix Reinii, deren Samen ich am 20. Juli 1920 auf dem Konseitôge in Nikko gesammelt hatte. Die Samen wurden am selben Tage in zwei Teile geteilt. Den einen Teil bewahrte ich über gemischtem Chlorcalcium und trocknem gelöschten Kalk auf, den zweiten verschloss ich dicht in ein Glasgefäss oder umwickelte ihn mit Paraffinpapier und liess ihn an der Luft liegen. Dieses Material wurde am 26. Juli in mein Laboratorium in Tokyo gebracht und dort für die Versuche verwendet. Am nächsten Tage, d. h. am 27. Juli, zeigten alle diese Proben mehr oder weniger Keimung, aber fünf Tage später zeigte es sich, dass der zweite Teil ganz zu Grunde gegangen war. Der erste Teil dagegen behielt bis ins nächste Jahr, d. h. nach noch 180 Tagen, einige Prozente seiner Keimfähigkeit. Ich konnte jedoch bei meinem Experiment mit S. Reinii keine besondere Widerstandsfähigkeit gegen Austrocknen bemerken, wie das Kinzel für Alpenweiden angibt.

In den folgenden Versuchen benutzte ich einen kleinen Eisschrank, welcher durch Eis von oben gekühlt wurde. Temperaturschwankungen waren natürlich nicht zu vermeiden, immerhin erzielte ich im heissen Sommer eine Temperatur von 10–13°C. Besondere Aufmerksamkeit wurde auch diesmal der Vorbehandlung der Samenernte geschenkt. Um mässige Luftfeuchtigkeit für Samen zu erzielen, bediente ich mich hauptsächlich der Schwefelsäure- und Chlorcalciumgemische.

Mein Versuchsmaterial war dasselbe wie vorher: S. Pierotii und S. japonica. Es wurde von denselben Bäumen gesammelt, welche mir 5 Jahre hindurch die Samen geliefert hatten. Die Bäume befinden sich im botanischen Garten der landwirtschaftlicher Fakultät der kaiserlichen Universität zu Tokyo.

Die Methode des Keimungsversuches, die Aufbewahrungsweise u. a. waren fast gleich wie vorher.

VERSUCH 1.

Am 4. Mai 1921 wurden gereifte Kätzchen von S. Pierotii und S. japonica gepflückt, im Zimmer getrocknet und am nächsten Tage die entblössten Samen gesammelt. Ich habe die Samen auf folgende vierfache Weise aufbewahrt.

1. Die Samen sind mit Paraffinpapier umwickelt und im dunkeln Zimmer aufbewahrt.
2. Die Samen sind in einer Glasflasche dicht verschlossen und im dunklen Zimmer aufbewahrt.

3. Die Samen sind mit Paraffinpapier umwickelt und im Eisschrank aufbewahrt.
4. Die Samen sind in einer Glasflasche dicht verschlossen und im Eisschrank aufbewahrt.

Jede oder jede zweite Woche wurde eine Probe entnommen und dem Keimungsversuch unterworfen. Das Resultat war folgendes.

TABELLE I.

Aufbewahrungsweise	Versuchstag*	12. V. (7)**				19. V. (14)				26. V. (21)				2. VI. (28)				9. VI. (35)				23. VI. (49)			
	Keimzustand / Name	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe
mit Paraffinp. umwickelt im Zimmer	S. Pierotii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
	S. japonica	0	0	***2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
im Gefäss dicht verschl. im Zimmer	S. Pierotii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
	S. japonica	0	6	78	84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
mit Paraffinp. umwickelt im Eisschrank	S. Pierotii	86	8	6	100	60	9	10	79	/	/	/	/	/	/	/	/	/	/	/	/				
	S. japonica	94	6	0	100	88	6	4	98	/	/	/	/	/	/	/	/	/	/	/	/				
im Gefäss dicht verschl. im Eisschrank	S. Pierotii	76	4	6	86	45	18	15	78	5	5	13	23	3	4	17	24	0	0	11	11	0	0	0	0
	S. japonica	94	6	0	100	95	4	1	100	57	14	21	92	44	14	32	90	58	23	9	90	12	15	28	55

* Unter Versuchstag ist der Tag verstanden, an dem der Keimversuch eingeleitet wurde.

** Die Ziffer in der Klammer bedeutet die Zahl der Aufbewahrungstage.

*** Die Zahlen in der Tabelle bedeuten des jeweilige Keimprozent, das immer eine Woche nach dem Versuchstag festgestellt wurde.

Wie die Tabelle zeigt, verlieren die Samen, welche mit Papier umwickelt oder dicht im Glas verschlossen und im dunklen Zimmer aufbewahrt wurden, nach einer Woche ihre Keimkraft fast vollständig. Das stimmt völlig mit den in meiner ersten Arbeit mitgeteilten Versuchen (Nakajima 1921 S. 23–25) überein. Aber die Samen, welche im Eisschrank aufbewahrt wurden, zeigten eine weit längere Keimfähigkeit. Auch zeigt sich, dass zwischen den zwei Arten der Weiden beträchtliche Unterschiede bestehen. Darauf habe ich schon in meiner letzten Abhandlung hingewiesen, aber bei dem Versuch mit im Eisschrank aufbewahrten Samen trat der Unterschied besonders auffallend hervor. Während S. Pierotii im diesem Versuche nach 28 Tagen 3% gute Keimung zeigt, weist S. japonica noch 44% auf; nach 35 Tagen hat jene keine Keimung, diese noch 58% gezeigt.

VERSUCH 2.

Ferner machte ich folgendes Experiment. Die gereiften Kätzchen von S. Pierotii wurden anfangs mit einen Gazestück umwickelt und dann direkt auf das Eis im Eisschrank gelegt. Von Zeit zu Zeit entnahm ich dieser Probe Kätzchen, trocknete sie einige Stunden im Zimmer, holte die Samen heraus und prüfte ihre Keimfähigkeit. Es ist selbstverständlich, dass bei dieser Behandlung die Samen vor dem Trocknen beständig feucht erhalten wurden.

Nachfolgende Tabelle zeigt das Ergebnis zweier Versuche; bei dem ersten wurden die Kätzchen am 1. Mai und bei dem zweiten am 19. Mai vom Baume gepflückt und der oben mitgeteilten Behandlung unterworfen.

TABELLE II.

S. Pierotii. Aufbewahrt direkt auf das Eis.

Versuchstag	11. VI.				8. VII.			
Keimung % / Beginn der Aufbew.	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe
I. 1. V.	54	21	2	77	32	11	35	78
II. 19. V.	71	17	5	93	35	15	30	80

Es hat sich also ergeben, dass die Lebensdauer der Samen noch mehr verlängert wird, wenn sie nicht bloss in den Eisschrank gelegt, sondern direkt aufs Eis gelegt werden. Im erstern Falle war die Keimungsfähigkeit schon nach 49 Tagen (siehe Tabelle I) verschwunden, während im letzteren noch nach 60 Tagen über 30% gute Keimung festzustellen war.

Da die Temperatur der Luft im Eiskasten nicht tiefer als 10°, die Eistemperatur fast 0° ist, so wird man wohl kaum mit der Annahme fehlgehen, dass in dem eben beschriebenen Experiment die niedere Temperatur massgebend für die Verlängerung des Lebens war.

VERSUCH 3.

Um zu erkennen, ob die Vorbehandlung, welche die Samen vor dem Sammeln erhalten, irgend einen Einfluss auf die Keimkraftdauer hat, machte ich folgende Versuche.

Die gereiften Kätzchen wurden, noch bevor sie sich am Baume geöffnet hatten, gepflückt und dann folgenden vier Vorbehandlungsarten (A-D) unterworfen,

A. Wurden im Freien bei Sonnenschein einen Tag lang getrocknet.
B. Vor dem Trocknen im Sonnenschein 18 Tage lang in der Luft des Eisschranks aufbewahrt.
.C. Vor dem Trocknen im Sonnenschein während einer Woche direkt aufs Eis gelegt, mit Gaze umwickelt.
D. Anstatt im Sonnenlicht bei Ofenwärme einen Tag lang getrocknet.

Die den vier Vorbehandlungsarten ausgesetzten Samen wurden sodann z. T. bei Zimmertemperatur im Finstern z. T. im Eisschrank gleichfalls im Finstern deponiert. Und die Aufbewahrungsweise war die folgende.

I. Bei Zimmertemperatur.
  1) Über reiner konzentrierter Schwefelsäure.
  2) Über mit gleichem Volumen Wasser gemischter Schwefelsäure.
  3) In der Glasflasche dicht verschlossen.
II. Im Eisschrank.
  1) Über reiner konzentrierter Schwefelsäure.

TABELLE III.

S. Pierotii.

		Versuchstag	27. V. (7)				10. VI. (21)				24. VI. (35)				9. VII. (50)				27. VII. (68)				11. IX. (114)				15. V. (360) (nächstes Jahr)			
		Keimung %	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe
Zimmertemperatur	1. Aufbew. über rein. Schwefelsäure	A	0	0	0	0	0	0	0	0	0	0	0	0																
		B	0	0	0	0	0	0	0	0	0	0	0	0																
		C	0	0	0	0	0	0	0	0	0	0	0	0																
		D	0	0	0	0	0	0	0	0	0	0	0	0																
	2. Aufbew. über Schwefelsäuregemisch	A	82	10	4	96	80	5	11	96	54	14	14	82	46	23	22	91	12	12	28	52	0	0	4	4	0	0	0	0
		B	16	6	8	30	15	18	5	38	9	5	9	23	4	16	5	25	0	1	6	7	0	0	2	2	0	0	0	0
		C	72	8	14	94	91	6	3	100	88	7	3	98	80	10	6	96	33	9	33	75	0	2	25	27	0	0	0	0
		D	90	4	0	94	46	10	38	94	57	18	18	93	46	8	25	79	20	11	20	51	0	5	8	13	0	0	0	0
	3. Zur Kontr. dicht verschlossen	A	0	0	92	92	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0								
		B	0	0	36	36	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0								
		C	0	0	90	90	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0								
		D	0	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								

Im Eisschrank	1. Aufbew. über rein. Schwefelsäure	A	0	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0												
		B	0	0	6	6	0	0	0	0	0	0	0	0	0	0	0	0												
		C	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0												
		D	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0												
	2. Aufbew. über Schwefelsäuregemisch	A	90	7	2	99	/	/	/	/	78	16	2	96	/	/	/	/	62	10	13	85	44	12	32	88	0	2	14	16
		B	31	5	4	40	/	/	/	/	21	9	10	40	/	/	/	/	17	8	8	33	24	10	8	42	0	0	10	10
		C	91	3	6	100	/	/	/	/	88	5	4	97	/	/	/	/	63	20	11	94	52	17	22	91	0	20	30	50
		D	90	4	2	96	/	/	/	/	70	16	9	95	/	/	/	/	43	12	30	85	35	15	35	85	0	5	18	23
	3. Zur Kontr. dicht verschlossen	A	82	10	8	100	1	7	20	28	0	0	0	0	0	0	0	0	0	0	0	0								
		B	16	4	10	30	2	6	30	38	0	0	0	0	0	0	0	0	0	0	0	0								
		C	80	12	8	100	2	6	48	56	0	0	0	0	0	0	0	0	0	0	0	0								
		D	12	22	32	66	0	3	6	9	0	0	0	0	0	0	0	0	0	0	0	0								

2) Über mit gleichem Volumen Wasser gemischter Schwefelsäure.

3) In der Glasflasche dicht verschlossen.

Die Tabelle III zeigt das Ergebnis.

Oder mit Worten ausgedrückt:

1. Die über reiner Schwefelsäure aufbewahrten Samen verlieren alle schon nach 1 Woche ihre Keimfähigkeit, nahe zu gleichviel, ob im Zimmer liegend oder im Eisschrank aufbewahrt.

2. Hingegen wird die Keimfähigkeitsdauer der über einem Gemisch von Wasser und Schwefelsäure liegenden Samen in hohem Grade verlängert, und in diesem Fall ist das Ergebnis im Eisschrank weit besser als im Zimmer.

3. Die Art der Vorbehandlung (A-D), der die Samen vor der verschiedenen Aufbewahrungsweise ausgesetzt wurden, hat grossen Einfluss auf die Lebensdauer der Samen. Wie die Tabelle zeigt, lieferte C das beste Ergebnis, dann folgt D und A nacheinander und schliesslich mit dem schlechtesten Erfolg B. Es behielten also die anfangs eine Woche hindurch auf Eis gekühlten dann in verschiedener Weise aufbewahrten Samen ihre Keimungsfähigkeit am längsten. Dann zeigte sich bei den über verdünnter Schwefelsäure im Eisschrank aufbewahrten Samen noch nach 114 Tagen 44% gute Keimung. Gewiss eine bemerkenswerte Tatsache. Es dünkt mich wahrscheinlich, dass durch die niedere Temperatur irgendeine stoffliche Veränderung im Embryo verursacht und die Widerstandsfähigkeit der Samen vermehrt wird. Diese Tatsache wird auch durch die folgenden Versuche bestätigt.

VERSUCH 4.

Das beim Versuche 2 benutzte Material, d. h. die Samen. welche vom 1. und 19. Mai bis 8. Juli auf dem Eis gekühlt worden waren, bewahrten ich über verdünnter Schwefelsäure im Eisschrank auf. Das Resultat zeigt die folgende Tabelle.

TABELLE IV.

S. Pierotii. Aufbewahrt über Schwefelsäuremischung im Eisschrank.

	Versuchstag	11. IX.				15. V. (im nächsten Jahr)			
	Keimung % / Aufbew. Weise	gute Keim.	schwache Keim.	schlechte Keim.	Summe	gute Keim.	schwache Keim.	schlechte Keim.	Summe
1. V.—8. VII. auf Eis gelegte Probe	Zimmertemp.	1	8	35	44	0	0	0	0
	im Eisschrank	31	15	42	88	2	15	30	47
19. V.—8. VII. auf Eis gelegte Probe	Zimmertemp.	10	12	30	52	0	0	0	0
	im Eisschrank	20	29	33	82	0	28	42	70

Wie die Tabelle lehrt, zeigen die Samen, welche während 68 Tagen auf Eis gekühlt und nachher über verdünnter Schwefelsäure im Eisschrank getrocknet wurden, noch nach 379 Tagen 2% gute Keimung.

VERSUCH 5.

Als Trockenmittel an Stelle von Schwefelsäure brauchte ich auch Chlorcalcium. Um mässige Luftfeuchtigkeit zu bekommen, benützte ich etwa 25 prozentiges wasserhaltiges Chlorcalcium. In diesem Versuche wurden alle Proben über diesem Mittel aufbewahrt und in den Eisschrank gebracht. Die Vorbehandlung der Samenernte war die folgende.

1. Die gereiften Kätzchen wurden während 12 Tagen (vom 1. Mai bis 12. Mai) in der Luft des Eisschranks aufbewahrt und dann einen Tag lang im Freien getrocknet.
2. Die gereiften Kätzchen wurden während 12 Tagen (vom 1. Mai bis 12. Mai) direkt auf dem Eis belassen und dann wie vorher behandelt.
3. Die Behandlung war dieselbe wie bei 2, doch blieben die Kätzchen nicht 12, sondern 24 Tage auf dem Eis.

Mit diesen so behandelten Samen wurden am 11. September desselben Jahres und am 15. Mai des nächsten Jahres Keimungsversuche eingeleitet.

TABELLE V.

S. Pierotii. Aufbewahrt über Chlorcalciummischung.

Art der Behandl.	Versuchstag 11. IX.				15. V. (im nächsten Jahr)			
	gute Keimung	schwache Keimung	schlechte Keimung	Summe	gute Keimung	schwache Keimung	schlechte Keimung	Summe
1	22	28	39	89	0	17	34	51
2	72	8	15	95	30	32	23	85
3	70	10	12	92	28	33	26	87

Der Versuch ergab gleich den zwei vorhergehenden Versuchen, das bemerkenswerte Resultat, dass die Samen, welche der Behandlung 2 ausgesetzt wurden, noch nach 1 Jahre nahezu 30% gute Keimung zeigten.

Zum Schluss will ich noch einen Versuch hinzufügen, der mich zufällig eine auffallende Tatsache lehrte. Bei dem Versuche 3 wurden unter anderen eine Probe in einem geschlossenen Glasgefäss über verdünnter Schwefelsäure im Eisschrank aufbewahrt. Während das andere Gefäss, jedesmal wenn für Keimungsversuche Samen entnommen wurden, von Zeit zu Zeit geöffnet wurde, blieb das eine immer verschlossen. Als ich nun auch Keimungsversuche mit den Samen, welche in dem stets verschlossen gebliebenen Gefäss sich befanden, machte, zeigte es sich, wie die nächste Tabelle VI lehrt, dass die Samen noch nach 360 Tagen zu 53% gut keimten, während die anderen, aus dem zeitweise geöffneten Gefäss entnommenen Samen, nach 114 Tagen nur noch zu 44% und nach 360 Tagen überhaupt nicht mehr keimten, wie wir das aus Tabelle III ersehen können.

Der Versuch lehrte auch gleichzeitig, dass sich die beiden verwendeten Arten S. Pierotii und S. japonica sehr verschieden verhalten. S. Pierotii erweist sich in unserem Falle viel lebenskräftiger als S. japonica.

TABELLE VI.

Aufbewahrt über Schwefelsäuremischung.

Versuchstag	15. V. (im nächsten Jahr)			
Keimung / Namen	gute Keimung	schwache Keimung	schlechte Keimung	Summe
S. Pierotii	53	27	13	93
S. Japonica	0	0	1	1

Nach dem Ausfall dieses Versuches muss man wohl annehmen, dass unter den gegebenen Umständen die mit der Öffnung des Gefässes verbundenen Lüftung und, dadurch plötzlich verursacht das einer veränderten Luftfeuchtigkeit und Temperatur Ausgesetztsein der Samen, welches zwar nur sehr kurze Zeit dauerte, aber doch öfters wiederholt wurde, verkürzend auf die Lebensdauer der Samen wirkt.

ZUSAMMENFASSUNG.

1. Die bisherigen, von verschiedenen Forschern (Wichura, Schröder, Wiesner, Woloszczak, Nohara und Kinzel) gemachten Untersuchungen über die Keimfähigkeitsdauer der Weidensamen haben im allgemeinen ergeben, dass diese eine verhältnismässig kurze ist. Die längste Dauer, welche bisher beobachtet wurde, beträgt nach Wiesner etwa 85 und nach Woloszczak 48 Tage. Die langjährigen, sich über 5 Jahre erstreckenden Versuche des Verfassers über die Keimfähigkeitsdauer von Weidenarten haben aber gezeigt, dass es durch eine bestimmte Behandlung und Aufbewahrung dieser Samen gelingt, ihre Lebensdauer und Keimfähigkeit auffallend viel länger, als bisher beobachtet wurde, zu erhalten, bei Salix Pierotii z. B. sogar 379 Tage.

2. Die Samen von Salix Pierotii und S. japonica verlieren ihre Keimfähigkeit unter gewöhnlichen Umständen schon nach einer Woche. Wenn sie aber gleich nach der Ernte in der Luft des Eisschranks gehalten oder noch besser, wenn die Kätzchen direkt auf das Eis gelegt werden, dann wird ihre Keimfähigkeit auffallend verlängert.

3. Die allerbesten Erfolge mit der Verlängerung der Lebensdauer

aber wurden erzielt, wenn die Kätzchen zuerst eine Woche auf Eis gelegt und dann die Samen über verdünnter Schwefelsäure (mit gleichem Volumen Wasser gemischt) oder wassergemischtem Chlorcalcium (Chlorcalcium 100 g. + Wasser 25 cc.) gehalten wurden.

4. Wenn Samen von Salix Pierotii im Eisschrank in zwei verschlossenen Glasgefässen über verdünnter Schwefelsäure aufbewahrt werden, jedoch mit dem Unterschied, dass das eine von Zeit zu Zeit gelüftet, das andere aber nicht geöffnet wird, so zeigt sich, dass das Unterbleiben der Lüftung die Keimfähigkeit auffallend verlängert; in einem bestimmten Fall keimten noch nach 360 Tagen 53% gut.

Zum Schlusse möchte ich Herrn Hofrat Professor Dr. Hans Molisch, der die Güte hatte, meine Arbeit vor der Drucklegung zu lesen, meinen herzlichsten Dank aussprechen.

LITERATURVERZEICHNIS.

1) Becquerel, P., Recherches sur la vie latente des graines. Ann. d. sci. nat. bot. 1907, S. 193.
2) Hildebrand, F., Über die Schutzeinrichtungen bei den Oxaliszwiebeln. Berichte. d. deutsch. bot. Ges. 2 Jg. 1884, S. 110.
3) Kinzel, W., Frost und Licht als beeinflussende Kräfte bei der Samenkeimung. Erläuterungen und Ergänzung zum ersten Buche. 1915, S. 27. Frost und Licht als beeinflussende Kräfte bei der Samenkeimung. Abschluss der Erläuterungen und Ergänzung zum ersten Buche. (Nachtrag II). 1920, S. 8.
4) Nakajima, Y., Seeds, irresistible to dryness, (Japanisch). The Bot. Mag. Tokyo. XXXIII. 1919. p. 169.
5) Nakajima, Y., On the life-duration of seeds of Salix (Japanisch). Ebenda. XXXV. 1921. p. 17.
6) Nobbe, F., Handbuch der Samenkunde. 1876, S. 368.
7) Nohara, S., On the germination of seeds of Salix, The Bot. Mag. Tokyo. XXVII. 1912, p. 23.
8) Oyama, M., Yamanarashi no Shushi Chozôshiken (Über die Aufbewahrung der Samen von Populus tremula var. villosa) (Japanisch). Ringyôshikenhôkoku. 9. 1911.
9) Oyama, M., Rinboku Shushi Chozôshiken (Über die Aufbewahrung der Samen der Forstbäume) (Japanisch). Ebenda. 10. 1913.
10) Oyama, M., Rinboku Shushi no Kansôdo to Hatsuga Hozonryoku to no Kankei (Über die Beziehung der Keimkraftdauer zu dem Trockengrade der Samen der Forstbäume) (Japanisch). Ebenda 21. 1920.
11) Pfundt, M., Der Einfluss der Luftfeuchtigkeit auf die Lebensdauer des Blütenstaubes. Jahrb. f. wiss. Bot. XLVII. 1910, S. 9.
12) Schröder, G., Über die Austrocknungsfähigkeit der Pflanzen. Untersuch. d. Bot. Inst.

Tübingen, II. 1886–1888, S. 8.

13) Shirasawa, Y., u. Inamura, T., Yamanarashi Naigi Yôsei Shiken. (Kulturversuche mit Sämlingen von Populus tremula var. villosa) (Japanisch). Ringyô Shiken Hôkoku, 5. 1908.

14) Shirasawa, Y., u. Oyama, M., Shiyô Rinboku Schushi no Chozôshiken. (Über die Aufbewahrung der Samen wichtiger Forstbäume) (Japanisch). Ebenda 8. 1910.

15) Wichura, J., Die Bastardbefruchtung im Pflanzenreich, erläutert an den Bastarden der Weiden. 1865, S. 2.

16) Wiesner, J., Biologie der Pflanzen. 2. Aufl. 1902, S. 57.

17) Woloszczak, E., Über die Dauer der Keimfähigkeit der Samen und Terminalknospenbildung bei den Weiden. Bot. Zentrbl. XXXIX. 1889. S. 150.

VORLÄUFIGE UNTERSUCHUNGEN ÜBER MYXOBAKTERIEN IN JAPAN.

Von

YOSHIJI YOSHII.

I. EINLEITUNG.

Obwohl die Entwicklungsgeschichte und die Morphologie der von Thaxter[1] entdeckten Myxobakterien sowohl durch die Untersuchungen dieses Forschers als auch durch die Arbeiten von Baur,[2] Quehl,[3] Vahle[4] und Kofler[5] genau bekannt geworden sind, sind doch ihre physiologischen und biologischen Eigenschaften nur gelegentlich berührt worden. Wer sich mit der Untersuchung der Myxobakterien beschäftigt, dem fallen sofort die Differenzen in der Farbe auf.

Bei der Untersuchung der Myxococcen, die in Japan häufig vorkommen, bin ich auch auf die Frage gestossen, ob man die Farbe der Myxococcen als ein für sie charakteristisches Merkmal ansehen dürfte, da die Fruchtkörper gewöhnlich intensiv rot, hellrot, gelbrot, orange und sogar gelb sind.

In seiner letzten Arbeit hat Thaxter (1904, p. 409) selbst erwähnt, dass die von ihm anfangs nach Farbtönen aufgestellen Arten: Myxococcus ruvescens und M. virescens dadurch nicht unterschieden werden können. Baur hat auf das Vorkommen der verschiedenen

---

[1] Thaxter R., On the Myxobacteriaceae, a new order of Schizomycetes. Bot. Gaz., 1892, Vol. XIV, p. 389.

——, Further observations on the Myxobacteriaceae. Bot. Gaz., 1897, Vol. XXIII, p. 395.

——, Notes on the Myxobakteriaceae. Bot. Gaz., 1904, Vol. XXXVII, p. 405

[2] Baur, E., Myxobacterienstudien. Arch. f. Protistenk., 1905, Bd. V, p. 92.

[3] Quehl, A., Untersuchungen über die Myxobakterien. Centralb. f. Bakt. Abt. II, 1906, Bd. XVI, p. 9.

[4] Vahle, C., Vergleichende Untersuchungen über die Myxobakteriazeen und Bakteriazeen, sowie die Rhodobakteriazeen und Spirillazeen. Centralb. f. Bakt. Abt. II, 1910, Bd. XXV, p. 178.

[5] Kofler, L., Die Myxobakterien der Umgebung von Wien. Sitzungsb. d. K. A. d. Wiss. Wien, math-naturw. Kl. Abt. I, 1913, Bd. CXXII, p. 845.

Farbtöne im Fruchtkörper der Myxococcen aufmerksam gemacht, ist jedoch nicht näher darauf eingegangen. Quehl (p. 22) untersuchte den Znsammenhang der verschiedenen Formen dieser fraglichen Myxococcen und kam zu dem Schluss, dass die beiden Arten immer durch abweichende Sporengrösse und Farbe der Fruchtkörper unterschieden sind. Kofler (p. 859), dem dieses eigenartige Verhalten auch aufgefallen war, fand sogar, dass der Unterschied in der Sporengrösse nicht immer so gross ist und dass es nicht selten Formen gibt, deren Zugehörigkeit zur einen oder anderen Spezies nicht zu entscheiden ist, da die Sporen auf der Grenze standen und die Farbe keinen Anhaltspunkt für die Erkennung beider Bakterien bot.

Nun ist es die Frage, ob die verschiedenartigen Formen in eine einzige Spezies einzureihen und die verschiedenen Farben auf veränderte Bedingungen zurückzuführen sind. Wenn diese beiden Formen verschiedener Art sind, so muss man sie durch charakteristische Merkmale voneinander abgrenzen und ausser dem Unterschied in der Farbe noch andere Eigentümlichkeiten irgendwelcher Art feststellen können. Die Aufgabe ist aber wohl nur mit Hilfe von Reinkulturen zu lösen. Wegen der Leichtigkeit der Reinzüchtung des Myxococcus ruvescens wurden seine physiologischen Eigenschaften ziemlich klar gelegt, aber beim Myxococcus virescens fehlt es an eingehenden Versuchen, da diese Bakterien schwer rein zu züchten sind. Mit geeignetem Nährboden gelang es mir, Myxococcus virescens rein zu züchten und seine physiologischen Eigentümlichkeiten mit denen von M. ruvescens zu vergleichen.

In vorliegenden Untersuchungen beschränkte ich mich daher hauptsächlich auf diese Aufgabe und führe die anderen morphologischen und systematischen Eigenschaften der Bakterien nur beiläufig an.

II. AUFKOMMEN DER MYXOBAKTERIEN IN JAPAN.

Während seines Aufenthaltes in Sendai hat Herr Prof. Hans Molisch eine Form von Myxococcus rubscens auf Pferdemist⁽¹⁾ gefunden, und er regte mich dazu an, die Myxobakterien in Japan zu untersuchen. Für seine freundlichen Ratschläge und seine Unterstützung spreche ich

---

1) Einige Pferdemistknödel wurden in eine Flasche gegeben und im Laboratorium stehen gelassen.

ihm auch an dieser Stelle meinen herzlichen Dank aus.

Um Myxobakterien zu gewinnen, habe ich Mist in eine Petrischale oder in ein grösseres Gefäss auf das zu benutzende Filtrierpapier gegeben, im Thermostaten bei 26°C und 36°C gehalten und bisweilen begossen, aber nicht so stark, wie Mist im freien Wasser liegt. Das Erhalten der richtigen Feuchtigkeit des Mistes ist für die Kulturen am wichtigsten und nötigsten. Frischer sowie auch zu alter und ausgetrockneter Mist ist nicht verwendbar, ziemlich lange Zeit im Freien liegender liefert dagegen immer ausgezeichnetes Material. Erhält man ihn nicht in günstigem Zustand, so entsteht bald ein dichter Überzug von Schimmelpilzen, Hutpilzen und Bakterien auf dem Mist, wodurch das Aufkommen von Myxobakterien ganz verhindert wird.

Die Optimumtemperatur für Myxobakterien beträgt nach Baur und Quehl (p. 30) 35°C. Es ist mir jedoch nicht geglückt, bei höherer Temperatur Myxobakterien zu gewinnen, bei niederer erhielt ich aber fast immer Myxococcus rubescens, öfters auch M. virescens.

Das Wachstum der Myxobakterien auf dem im Jahre 1923–1924 untersuchten Miste ist im Folgenden zusammengefasst:[1]

TABELLE I.

In der Tabelle bedeutet + Fruchtkörperbildung.

Mist Myxobak.	Hasen	Hirsche	Pferde	Affen	Bären	Verfallende Wurzelstück
Myxococcus ruvescens	++	++	+	+	−	+
Myxococcus virescens	+	+	−	−	+	−

Auf Mist von Hunden, Kühen und Katzen war nichts zu finden.

Hasen- und Hirschemist erwiesen sich als besonders ergiebige Fundorte für Myxococcen, und zwar habe ich immer auf dem Mist, wenn auch aus ganz verschiedenen Orten, z. B. auf dem Hirschemist aus Matsushima, aus Kinkwazan und aus Nara; dieselben Bakterien gefunden. Auf einem verfallenden Wurzelstück, das im Laboratorium liegen gelassen wurde, fand ich einmal eine grosse Menge Fruchtkörper von Myxococcus ruvescens, aber niemals auf Flechten, die nach Thaxter auch Fundorte von Myxococcen sein sollen.

---

[1] Wäre Mist von verschiedenen Arten noch genauer durchsucht worden, so hätte man noch viele Arten von Myxobakterien finden können, aber ich wollte ja keine systematische Untersuchung dieser Bakterien anstellen.

Die häufigsten Formen der Myxobakterien in Japan sind, wie obige Tabelle erkennen lässt, Myxococcus ruvescens und M. virescens, obwohl letzterer viel seltener sich findet. In Bezug auf die Häufigkeit der Species machen sich in Nordamerika und Europa erhebliche Unterschiede bemerkbar, jedoch ist Myxococcus ruvescens (Myxococcus ruber, Baur)[1] immer die häufigste Form. Dies gilt auch von Japan. Woraus zu ersehen ist, dass Myxococcus ruvescens kosmopolitische Myxobakterien sind.

III. REINZÜCHTUNG.

Um Reinkulturen von Myxobakterien zu erzielen, haben viele Forscher verschiedene Methoden beschrieben, es gibt aber kein ganz befriedigendes Verfahren. Das gewöhnliche Plattengussverfahren ist für die Reinzüchtung von Myxococcus ruvescens anwendbar, aber man kann damit bei M. virescens nicht zum Ziel kommen. Ich empfehle den dichten Mistagarnährboden nach Vahle (p. 186), auf dem auch leicht eine grosse Menge von Fruchtkörpern des Myxococcus virescens gebildet wird. Von diesen Rohkulturen ausgehend, kann man weiter zur Reinzüchtung fortschreiten.

Leichter und sicherer gelangte ich aber zu Reinkulturen von Myxococcen nach folgendem Verfahren. Impft man zunächst die Bakterien unmittelbar auf den Mist, der auf Watte im Reagenzglas bereits sterilisiert wurde, so wird unter günstigen Umständen bald ein Kranz von Fruchtkörpern gebildet, ohne dass sich dabei kräftiges Wachstum des vegetativen Schwarms zeigt. Solche Fruchtkörper sind gewöhnlich noch mit verunreinigenden Bakterien gemischt, aber das hat den Vorteil, dass man die Myxobakterien längere Zeit aufbewahren kann. Nun strich ich die Fruchtkörper auf die Oberfläche des Nähragars aus. Auf geeignetem Nährboden zeigt der Schwarm schwaches Wachstum, aber die Fruchtkörper kommen kräftig vor,[1] insbesondere am oberen Teil des Nährbodens, wo das Agar allmählich ausgetrocknet wird. Nach Vahle (p. 196) hindert die damit erzielte Trockenheit des Nährbodens das Wachstum der verunreinigenden Bakterien, aber nicht die Ausbreitung der Myxobakterien. Je weiter daher letztere wachsen, um so weniger sind erstere ihnen beigemischt. Von dieser am Rande ziemlich reinen Stelle wurden Myxobakterien wieder abgeimpft und dies so oft wiederholt, bis die Form rein auf Agar wuchs. Das oben erwähnte Verfahren wird nur dann mit gutem Erfolg ausgeführt, wenn man die Myxobakterien auf den Nährböden zur Fruktifikation bringen kann.

---

[1] Baur nannte die von ihm rein kultivierte Form Myxococcus ruber. Quehl (p. 18) betrachtet sie aber als eine der vielen Rassen von M. ruvescens.

Viele Forscher suchten zwar ein Nähragar zu finden, auf dem Myxobakterien wachsen und fruktifizieren, wie auf ihrem natürlichen Substrat, kamen aber im allgemeinen noch zu keinem befriedigenden Resultat. Als bestes Substrat für Myxococcus ruvescens wird allgemein ein Kartoffeldekokt angegeben; man kann darauf bei Kulturen von Myxobakterien einen guten Erfolg erzielen, wie aus folgender Tabelle sich erkennen lässt. In diesem Versuche wurde M. ruvescens auf verschiedenen Nährböden bei 26°C kultiviert und seine Fruchtkörperbildung besonders ins Auge gefasst.

TABELLE II.

Dekokt	Alter der Kultur; Tage:					
	3	4	5	10	26	42
Glysine hispida, Spross	Kol. unsichtbar	—	—	Kol. nur ausgedehnt	—	Frk. nicht gebildet
Heu	Kol. kräftig	—	—	Frk. viele	Frk. gross und braun	Frk. verblasst, abgeplattet
Kartoffel	Kol. klein	—	Frk. gebildet	Frk. viele	Frk klein und zahlreich	Frk. verblasst, etwas abgeplattet
Tofu-rückstand	Kol. unsichtbar	Frk. gebildet	—	Frk. viele	Frk. sehr viele	Frk. rot
Daucus Carota, Wurzel	Kol. klein von 2 mm. Breite	—	—	Frk etw. viele	Frk. viele	Frk. verblasst
Allium Cepa, Zwiebel	Kol. ausgedehnt	—	—	Frk. wenig	Frk. viele	Frk. verblasst

Kol. = Kolonie, Frk. = Fruchtkörper.

---

[1] Wie Vahle (p. 212) und andere Forscher festgestellt haben, wird die Fruchtkörperbildung auf Substrat mit reichlichem Nährstoffgehalt unterdrückt, und es wächst nur vegetativ kräftig.

Fruchtkörper werden auf verschiedenem Nähragar gebildet, die Geschwindigkeit der Ausbildung ist je nach der Art der Nährböden deutlich verschieden; während die Fruchtkörper auf Tofu1)- und Kartoffeldekoktagar schon innerhalb 5 Tage sich zeigen, kommen sie auf den anderen Nährböden, mit Ausnahme von Grysinedekoktagar, erst nach 10 Tage vor, und nach 6 Wochen ist die rote Farbe der Fruchtkörper verblasst und ihre kugelige Form etwas abgeplattet. Der Stäbchenschwarm verhielt sich ganz verschieden; er wächst kräftig auf Heudekoktagar, jedoch auf Kartoffeldekokt- und Tofuagar zeigt er schwaches Wachstum.

Es ist hervorzuheben, dass die Fruchtkörper auf dem Tofuagar, wie auf natürlichem Substrate, nach mehr als einem Monat beständig bleiben und ihre rote Farbe noch erhalten, während sie auf den anderen Nährböden nach einigen Wochen in sich zusammenfliessen. Das zeigt also, dass Tofuager für Myxococcus ruvesçens das beste Substrat ist. Ich werde später wieder darauf zurückkommen.

Myxococcus virescens verhielt sich bei den einzelnen Nährböden ganz verschieden. Ich habe mit M. virescens fast die gleichen Versuche angestellt wie mit M. ruvescens. Er wächst vegetativ weniger als M. ruvescens auf den verschiedenen Nährböden, und Fruchtkörper treten bei ihm nicht auf. Fruchtkörperbildung findet sogar nicht auf Kartoffeldekoktagar statt, das sich für M. ruvescens, als ausgezeichneter Nährboden erwiesen hat. Impft man Fruchtkörper von M. virescens auf Kartoffeldekoktagar über, so dehnt sich eine Kolonie kräftig aus, die aber zum Teil aus verunreinigenden Bakterien besteht, welche jene schliesslich überwuchern, wie dies bei Rohkulturen gewöhnlich der Fall ist. Nicht nur nicht mit Kartoffeldekoktagar, sondern auch mit andern Nährböden glückte es mir nicht, Fruchtkörperbildung des M. virescens zu erzielen. Mit einem spezifischen Nährboden gelang es mir leicht, ihn zur Fruktifikation zu bringen. Auf einem Nährboden (Tofuagar, der durch Abkochen von Bohnengallerte(Tofu)-Rückstand hergestellt war,2)) wächst er vegetativ schwach,

---

1) Vgl. hierzu Fussnote 2.

2) Zur Herstellung von Tofu (Bohnengallerte, ein gewöhnliches Nahrungsmittel in Japan) werden Sojabohnen zunächst zerrieben und abgekocht, dann ausgepresst. Der Rückstand wird Tofukara (Bohnengallertrückstand) genannt, dessen man sich auch als eines guten Nahrungsmittels für Pferde, Kaninchen, Kühe u. s. w. bedient.
In vorliegender Arbeit nannte ich den mit diesem Rückstand hergestellten Nähragar der Kürze halber *Tofuagar*.

aber Fruchtkörperbildung zeigt sich sehr schnell und sicher. Das sei an einem Beispiel auseinandergesetzt.

TABELLE III.

In der Tabelle bedeutet + Fruchtkörperbildung.

Nährboden Myxobak.	Kartoffeldekoktagar			Milchagar			Tofuagar			Hirschemistagar		
M. ruvescens (Stamm II)*	—	+	++ braun	—	++ faltenförmig	++ verblasst	+	++	+++	—	++	++ abgeplattet
M. ruvescens (Stamm I)	—	—	+	+	+	++ faltenförmig	—	++	+++	—	—	—
M. virescens (Stamm V)	—	—	++	—	—	++	—	++ klein	+++	—	++	++ braun
M. virescens (Stamm IV)	—	—	—	—	+	++ zerfliessen	—	++ klein	+++	—	—	++
Kulturtage	3	8	15	3	8	15	3	8	15	3	8	15

* Vgl. über dem Stamm die Angaben auf p. 289.

Aus obiger Angabe lässt sich erkennen, dass Tofuagar für Myxococcus virescens sowohl als auch für M. ruvescens in Reinkulturen der beste Nährboden ist. Man kann darauf den schwer züchtbaren M. virescens leicht zur Fruchtkörperdildung auch in Rohkulturen bringen. Davon ausgehend, konnte ich mit dem oben auf p. 280 erwähnten Verfahren ihn rein gewinnen. Dass dieser Nährboden sich auch für Myxococcus ruvescens als ausgezeichnet erweist, wurde bereits oben gesagt (p. 282).

IV. VERHALTEN DER MYXOBAKTERIEN AUF VERSCHIEDENEN NÄHRBÖDEN.

Im Folgenden will ich mich weiter mit dem Verhalten der beiden Bakterien auf verschiedenen Nährböden beschäftigen, mit besonderer Rücksicht auf die Verschiedenheit sowohl ihrer physiologischen als auch ihrer biologischen Eigentümlichkeiten.

Gelatine wird durch Myxococcus ruvescens leicht verflüssigt, aber durch M. virescens nicht. Impft man diese Bakterien z. B. auf Bouillongelatine, so wächst das vegetative Stäbchen von M. virescens kräftiger als das von M. ruvescens, ohne dabei den Nährboden zu verflüssigen. Die Verflüssigung der Gelatine lässt sich dagegen bei M. ruvescens innerhalb einiger Tage deutlich erkennen. Der ausgeschiedene Farbstoff der Bakterien ist auch verschieden. Der gelbliche Farbstoff von M. virescens zeichnet sich dadurch aus, dass er eine auffallende Fluorescenz zeigt. Im durchfallenden Licht ist er gelb, im auffallenden Licht schön grün. Dieser fluoreszierende Farbstoff bildet sich besonders in peptonhaltigen Nährböden.[1] Bei den Kulturen von M. ruvescens scheidet ein hellroter Farbstoff aus, der in Lösung keine Fluorescenz zeigt.

Eine noch auffallendere Erscheinung ist die Agarlöslichkeit des M. ruvescens, die M. virescens ganz fehlt. Die Bakterien, welche die Eigenschaft besitzen, Agar zu verflüssigen, sind nur wenig bekannt.[2] Vahle hat bereits bei Kulturen von M. ruvescens diese Erscheinung beobachtet, ist aber nicht näher darauf eingegangen, hat nur angegeben, dass die Kolonie des Myxococcus ruvescens auf Agarnährboden so in den Agar hineinwächst, als ob das Agar an der betreffenden Stelle eingeschmolzen wäre (p. 188). In der Tat kann man diese Erscheinung leicht bei M. ruvescens sehen, der auf den Nähragar in Punktform geimpft worden war.

Die beiden Bakterien verhalten sich auf Milchagar ganz verschieden, und zwar kann man sie nur dadurch voneinander unterscheiden. Impft man M. ruvescens auf Michagar in Punktform, so dehnt sich die Kolonie kreisförmig aus, die ganz dünn und zart, ja kaum wahrnehmbar ist, während der Nährboden durchlässig wird. Ein Beweis, dass proteolytische Enzyme vorhanden sind.[3] Behandelt man M. virescens in ganz gleicher Weise auf Milchagar, so bildet der Schwarm darauf einen weissen Belag, der aber allmählich gelb wird, ohne aber proteolytische Erscheinungen zu erzeugen. Die gelegentlich mit Milch oder Milchagar angestellten Versuche zeigen ferner, dass die Labenzyme vielleicht nur in M. virescens vorhanden sind.

---

[1] Nach Ackermann (Arch. f. Hyg. Bd. 73, p. 14) bleibt die Pigmentbildung des Bact. prodigiosus auf eiweisshaltigem, festem Nährboden aus: das Verhältnis ist also hier umgekehrt.

[2] Vgl. hierzu H. und E. Pringsheim, Über die Verwendung von Agar-Agar als Energiequelle zur Assimilation der Luftstickstoffes (Centralb. f. Bakt. Abt. II, 1910, Bd. XXVI. p. 227) und dort weitere Literatur.

[3] Vgl. hierüber Eijkmann, Über Enzyme bei Bakterien und Schimmelpilzen. Centralb. f. Bakt. 1901, Bd. XXIX, p. 841.

V. FLÜSSIGE NÄHRMEDIEN.

Bei der Anwendung der Dekokte für die Beurteilung ihres Nährwertes ist es nötig, eine bestimmte Konzentration zu verwenden. Deshalb verfahre ich zur Herstellung der Dekokte stets nach Vahle (p. 180) folgendermassen: ich koche immer je 5 g des Materials mit 100 ccm Leitungswasser eine halbe Stunde, filtriere es und bringe es wieder auf das Volumen von 100 ccm.

Da ich oben (p. 281) das Verhalten des M. ruvescens auf den verschiedenen Nähragarböden, die aus diesen Dekokten hergestellt wurden, beschrieben habe, so will ich mich hier nur mit den Versuchen mit M. virescens in den verschiedenartigen Dekokten beschäftigen.

Wenn man M. virescens in der Abkochung impft, so entsteht zunächst auf der Oberfläche der Flüssigkeit eine Haut, die aus Stäbchen besteht. Gleichzeitig findet fast immer Trübung der Lösung und dann Niederschlag einer flockigen Masse in der Lösung statt. Endlich tritt wieder Klarheit der Lösung ein, die längere Zeit klar bleibt. Bezüglich dieser charakteristischen Erscheinung haben Vahle und Kofler auch darauf hingewiesen, dass das Klarbleiben der Lösung als Zeichen für die Reinheit der Myxobakterien gelten darf. Nach längerer Zeit wird ein Kranz von Fruchtkörpern auf der Haut, insbesondere an ihrem Rande, entlang der Glaswand gebildet.

Mit Ausnahme vom Allium-Dekokt findet Fruchtkörperbildung in allen bereit angegebenen Dekokten statt, besonders deutlich im Heu- und Daucus-Dekokt. Das beste Resultat erhielt ich aber auch hier beim Tofu-rückstand-Dekokt.

Nun will ich auf das Verhalten der beiden Bakterien in der bekannten Zusammensetzung eingehen.

Bei der Erörterung über den Einfluss des Nährbodens auf Myxobakterien hat Quehl (p. 29) darauf hingewiesen, dass ein geringer Zusatz von Pepton, etwa 1/2 Proz., zu Mistagar ein sehr kräftiges Wachstum mit regelmässiger Bildung von Fruchtkörpern ergibt, aber bei M. virescens schon durch geringe Menge Pepton die Fruchtkörperbildung unterdrückt wird. Vahle (p. 182) empfiehlt als günstige

Nährlösung für M. ruvescens 0,2%ige Peptonlösung. Es ist möglich, dass sich die beiden Bakterien gegenüber dem Peptongehalt der Nährlösung ganz verschieden verhalten.

Um einen Überblick über die Ernährungsverhältnisse der Bakterien zu geben, habe ich mit folgenden vier verschiedenen Nährlösungen Versuche angestellt;

1. Lösung mit 0,6 Proz. Pepton und 4 Proz. Rohrzucker.
2. Lösung mit 0,6 Proz. Pepton und 0,6 Proz. Rohrzucker.
3. Lösung mit 0,1 Proz. Pepton und 4 Proz. Rohrzucker.
4. Lösung mit 0,1 Proz. Pepton und 0,6 Proz. Rohrzucker.

Zusatz von 0,1 Proz. $MgSO_4$ und 0,1 Proz. K_2HPO_4 zu jeder Lösung.

Es zeigt sich dabei, dass M. rubescens in peptonreicher Lösung, besonders bei Zusatz von grosser Menge Zucker vegetativ gut wächst, aber in Lösung von geringer Menge Pepton und Zucker nur kümmerlich, hier jedoch Fruchtkörperbildung kräftig stattfindet. M. virescens wächst auch in peptonreicher Lösung vegetativ sehr stark, und Fruchtkörper werden in allen 4 Lösungen gebildet. Während die Farbe der Lösung von einer geringen Menge Pepton und von Zucker fast farblos bleibt, werden die übrigen 3 Lösungen nach einiger Zeit intensitiv gelb. Das beste Resultat bez. Fruchtkörperbildung erhielt ich bei M. ruvescens in der zwiten (peptonreich und zuckergering) und bei M. virescens in der dritten (peptongering und zuckerreich). M. rubescens ist daher eine Pepton mehr liebende Spezies als M. virescens. Hier sei daran erinnert, dass proteolytische Enzyme nur in Myxococcus ruvescens ausgeprägt erzeugt werden.

VI. REAKTION DES NÄHRBODENS.

Bezüglich der Fruchtkörperbildung darf man auch die Reaktion des Nährbodens nicht ausser Acht lassen. Über die Wirkung der Saüre auf die Myxobakterien haben wir nur wenige Arbeiten. Kruylff[1] gibt für Myxococcus javaensis an, dass die Sporenbildung durch geringe Menge von Säure oder Alkalien unterdrückt wird. Der gleiche Versuch wurde von Vahle (p. 223) mit M. ruber angestellt. Er fand, dass M. ruvescens viel mehr Alkalien als Säure verträgt und keine

---

[1] Kruylff, E., Die Lebensgeschichte von Myxococcus javanensis sp. n. Centralb. f. Bakt. Abt. II, 1908, Bd. XXI, p. 385.

Fruchtkörperbidung beim Säurezusatz stattfindet und nur schwache vegetative Entwicklung erfolgt.

Im Folgenden wird die Wirkung der Reaktion des Nährbodens auf die beiden Bakterien miteinander verglichen. Die füt Myxobakterien geeigneten Nährböden, Tofu- und Kartoffeldekoktagar, wurden zum Versuch verwendet und die Reaktion des Nährbodens durch Zusatz von Monokaliumphosphat oder Kalziumkarbonat hervorgerufen. Bezüglich der Fruchtkörperbildung haben die Versuche ergeben, dass es sich nicht nur um die Reaktion des Nährbodens, sondern auch um dessen Eigenschaften handelt. Myxococcus ruvescens bildet Fruchtkörper auf Kartoffeldekoktagar nur bei alkalischer Reaktion, aber auf Tofuagar auch bei saurer Reaktion, und zwar findet hier Fruchtkörperbildung kräftiger statt. Myxococcus virescens verhielt sich aber ganz anders; Fruchtkörperbildung kommt bei saurer Reaktion nicht nur auf Tofuagar, sondern auch auf Kartoffeldekoktagar vor, und bei alkalischer Reaktion erfolgt die Fruchtkörperbildung schwach, obwohl er vegetativ kräftig wächst. Daraus ergibt sich, dass die Wirkung der Reaktion auf die Fruchtkörperbildung in den beiden Bakterien ganz verschieden ist und dass daran auch die Eigentümlichkeit des Nährbodens teilnimmt. Es ist noch hervorzuheben, dass Zusatz von saurem Salz zum Tofuagar auf die Fruchtkörperbildung beider Bakterien eine sehr günstige Wirkung ausübt, und zwar ist in dieser Lösung schon nach 3 Tagen eine grosse Menge von Fruchtkörpern des schwer züchtbaren M. virescens zusehen.

VII. MODIFIKATION DER FARBE DER FRUCHTKÖRPER.

Wie bereits erwähnt, ist der Farbton der in Rede stehenden Bakterien ganz verschieden. Ich habe nicht nur die Verschiedenheit der Farbe der Fruchtkörper auf Mist, sondern auch ihre Farbveränderung in Reinkulturen beobachtet. Bezüglich der letzt erwähnten Erscheinung will ich im Folgenden einige wichtige Beispiele geben. Ein roter Myxococcus ruvescens, der auf Milchagar in Punktform geimpft wurde, zeigte schon am nächsten Tag an dieser Stelle proteolytische Reaktion. Die hellrote Farbe der Kolonie veränderte sich allmählich ins gelbe, und nach 2 Wochen bildeten sich einige gelbe Fruchtkörper am Rande der Kolonie.[1] Von einer jungeren Kolonie, deren Farbe noch rot war, habe ich Stäbchen auf Tofu-, Kartoffeldekoktagar und Milchagar geimpft. Während die Farbe der damit erzielten Kolonie auf den beiden ersten Nähragaren rot blieb, wurde sie auf Milchagar allmählich gelb. Wenn diese Kolonie ganz gelb geworden war, impfte ich ihre Stäbchen wieder auf Milchagar über, woraus sich auch eine gelbe Kolonie ergab, die aber keine Fruchtkörper erzeugte. Ich konnte aus diesen Stäbchen aber rote Fruchtkörper auf Mist erhalten.

Nun ein anderes Beispiel mit einem gelben Myxococcus virescens. M. virescens, der auf Milchagar geimpft worden war, zeigte schon am 5. Tage eine schöne, gelbe Kolonie.

Von dieser Kolonie wurden Stäbchen auf Milchagar überimpft, wobei sich gelbe Fruchtkörper bildeten, deren Sporen dann auf saures Tofuagar überimpft wurden. Nun dehnte sich zunächst eine rote Kolonie aus, deren Rand allmählich einen gelblichen Belag bekam. Im Inneren der Kolonie erzeugte sich aber ein Kranz von roten Fruchtkörpern. Nach Impfung dieser roten Sporen auf Mist konnte ich wieder die anfänglich gelben Fruchtkörper gewinnen.

Aus den vorstehenden Beispielen lässt sich erkennen, dass die rote und gelbe Farbe der Myxococcen sich je nach dem Nährboden modifizieren kann. Überblickt man die Resultate dieser Versuche, so bemerkt man sofort, dass Milchagar immer an der Farbveränderung teilnimmt und die Bakterien ihre anfängliche Farbe auf Mist wieder zurückgewinnen können. Die Ursache für diese Erscheinung müssen weitere Versuche klären.[2] Ich will hier noch ein Beispiel geben, um dafür einen Anhaltspunkt zubieten. Kultiviert man M. rubescens in den Nährlösungen von bekannter Zusammensetzung, so zeigen sich seine Farbtöne als verschieden; beim Zusatz von reichlich Pepton wird, ohne Rücksicht auf die Zuckermenge, die Farbe gelblichorange, während sie in Lösung von geringer Menge Pepton immer rot ist.

---

[1] Impft man die Bakterien auf Plattenagar in Punktform, so dehnt sich die Kolonie meist kreisförmig aus, und es wird ein regelmässiger Hexenring von Fruchtkörpern gebildet, worauf schon Quehl (p. 30) hingewiesen hat.

[2] Vgl. hierzu die Angaben von F. Wolf (zit. nach Zentralb. f. Bakt. Abt. II, 1910, Bd. XXVI, p. 248), er betrachtet die Farbveränderung auch als unvererbbare Modifikation und weist unter anderem darauf hin, dass die rote Farbe bei M. virescens durch Phenol- od. Chromatzusatz hervorgerufen wird, die gelbe Farbe bei M. ruvescens aber auf unbekannte Gründe zurückzuführen ist.

Aus dieser Tatsache ist zu vermuten, dass der Peptongehalt der Lösung dabei eine Rolle spielt.

VIII. RASSE DES MYXOCOCCUS RUBESCENS UND M. VIRESCENS.

Quehl durchsuchte systematisch die Umgebung von Berlin nach Myxobakterien und hat eine eingehende Untersuchung darüber veröffentlicht. Er teilte Myxococcus ruber in 8 Sippen (p. 22) und erforschte deren morphologische und physiologische Eigenschaften.

In vorliegender Untersuchung habe ich nicht die verschiedenen Spezien und Rassen der Myxobakterien zu finden gesucht, wohl aber während dieser Versuche drei Rassen von Myxococcus rubescens und zwei von M. virescens rein gezüchtet. Diese kamen aus verschiedenen Fundorten und besassen etwas voneinander verschiedene Eigenschaften, wie im Folgenden auseinandergesetzt werden wird.

I. M. rubescens, der auf Hasenmist gefunden wurde; er stimmt mit der von Thaxter aufgestellten Form beinahe überein, und ich verwendete diesen Stamm zum vorliegenden Versuch mit M. ruvescens.[1]

II. Der Stamm, welchen ich auf Affenmist gefunden habe, hatte auf Mist eine braunrote Farbe; nach Reinzüchtung bildeten sich rot gefärbte Fruchtkörper. Er ist nicht scharf von I. unterscheidbar.

III. Der Stamm kam auf dem Wurzelstück einer Tanne vor, wie bereits auf p. 280 erwähnt. Fruchtkörper sind etwas kleiner als die der vorigen Bakterien, zeichnen sich aber dadurch aus, dass die Farbe schön hellrot und die Sporen kleiner als die der gewöhnlichen Spezies sind.

IV. M. virescens, der auf Hirschemist gefunden war, stimmte ziemlich gut mit Thaxter's Beschreibung überein, sein vegetatives Stäbchen wich aber etwas ab; und zwar war es kürzer, ja viel kürzer als das von Quehl (p. 18) beschriebene; jedoch möchte ich ihn als eine Rasse von M. virescens betrachten. Ich verwandte diesen Stamm zum Versuche.[2]

V. Der Stamm, welcher auf Mist von einem gehaltenen Bär gefunden war, hat kleinere Fruchtkörper von schön gelber Farbe, seine Sporen weichen aber nur wenig von denen von IV. ab.

---

[1] Die vegetativen Stäbchen sind 3,5–5,0 μ lang, 0,4 μ breit, die Sporen messen 1,0–1,2 μ.

[2] Stäbchen, 0,6–2,0 μ lang, 0,5 breit, Sporen 1,5–2,1 μ.

Ich habe keine näheren Untersuchungen über die spezifischen Eigentümlichkeiten dieser Rasse angestellt, jedoch zeigen sie in ihrer Ernährung schon deutliche Spezialität, indem eine Rasse auf einem Nährboden Fruchtkörper ausbildet, aber auf anderen nur vegetativ wächst.

Diese Verhältnisse veranschaulicht folgende Tabelle.

TABELLE IV.

In det Tabelle bedeutet + Fruchtkörperbildung.

Nährboden Myxobak.	Hirschemist-agar	Kartoffel-dekoktagar	Tofuagar	Milchagar	Stamm gefunden auf:
Myxococcus rubescens (Stamm I.)	—	+	+++	++	Hasenmist
M. rubescens (Stamm II.)	++	++	+++	++	Affenmist
M. rubescens (Stamm III.)	—	+	—	—	Wurzelstück
Myxococcus virescens (Stamm IV.)	++	—	+++	+	Hirschemist
M. virescens (Stamm V.)	++	++	+++	++	Bärenmist

Daraus ergibt sich, dass eine Rasse auf dem Nährboden, von dem sie stammt oder dessen Zusammensetzung ihrem gefundenen Substrat nahe steht, leicht und reichlich Fruchtkörper erzeugt.

Obwohl die Erklärung dieser Erscheinung noch weitere, eingehende Versuche erfordert, kann man jedenfalls schon mit einigem Recht sagen, dass die vorhandenen Differenzen zur Aufstellung der Rasse ausreichen.

IX. ZUSAMMENFASSUNG.

1) Die häufig vorkommenden Myxobakterien in Japan sind Myxococcus ruvescens und M. virescens. Hasen- und Hirschemist erweisen sich als ergiebigste Fundorte. Die Optimum-temperatur für Kulturen dieser Bakterien ist niedriger, als allgemein angenommen wird, und beträgt etwa 26°C.

2) In vorliegender Arbeit verglich ich die physiologischen Eigentümlichkeiten dieser beiden Bakterien. Während Myxococcus ruvescens leicht gezüchtet werden kann, ist die Reinkultur von M. virescens schwer, und zwar kann man ihn auf Kartoffeldekoktagar nicht rein bekommen.

3) Für Kulturen von M. virescens habe ich einen spezifischen, ergiebigen Nährboden gefunden, auf dem M. virescens gut und leicht Fruchtkörper bildet. Dieser Nährboden erweist sich auch für M. ruvescens als bester Nährboden.

4) M. ruvescens verflüssigt Gelatine, M. virescens aber nicht. M. virescens zeichnet sich dadurch aus, dass er auf peptonhaltigem Nährboden fluorezierenden, gelben Farbstoff ausscheidet. M. ruvescens zeigt aber die bemerkenswerte Eigenschaft, Agar zu verflüssigen, die M. virescens fehlt. Das Verhalten der beiden Bakterien auf Milchagar ist besonders nennenswert; impft man M. ruvescens in Punktform auf diesen Nährboden, so zeigt sich die proteolytische Wirkung deutlich, dagegen entwickelt sich bei M. virescens zunächst ein weisser Belag auf der Kolonie, wobei kaseinfällende Wirkung zu beobachten ist.

5) Die beiden Bakterien entwickeln sich gut in Flüssigkeiten von bekannter Zusammensetzung, und meist tritt Fruchtkörperbildung ein. M. ruvescens ist eine Pepton mehr liebende Spezies als M. virescens.

6) Die Reaktion des Nährbodens wirkt auf die beiden Bakterien ganz verschieden; M. virescens kann viel stärkere saure Reaktion des Nährbodens vertragen als M. ruvescens, und zwar findet Fruchtkörperbildung in schwach saurem Nährboden kräftig statt.

7) Die Farbe der Fuchtkörper der beiden Bakterien kann sich auch in Reinkulturen verändern, aber sie lässt sich auf bestimmtem Nährboden wieder zurückgewinnen. Wahrscheinlich spielt der Peptongehalt des Nährbodens dabei eine Rolle.

8) Wir möchten die Farbe der Myxococcen noch als ein charakterisches Merkmal ansehen, obwohl sie aus unbekannten Gründen modifiziert werden kann. Auf Grund der voneinander abweichenden Unterschiede in den physiologischen Eigentümlichkeiten kann man sicher die beiden Spezies Myxococcus ruvescens und M. virescens, Thaxter beibehalten.

EFFECT OF LIGHT ON THE MOVEMENTS OF THE EARTHWORM, ALLOLOBOPHORA FŒTIDA (SAV.).

By

EKITARO NOMURA.

The present investigation was undertaken at the suggestion of Prof. G. H. Parker and was carried on in the dark room of his laboratory in Harvard University, during the year 1923 while I was staying in Cambridge, Massachusetts, U. S. A. The writer wishes to express his deep obligation to him for the free use of the dark room and for his deep interest and for the encouragement given during the progress of the investigation.

The report consists of the following nine chapters:

Chapter I. The differential influence of light upon fresh and tired worms.
Chapter II. The average limit of the free or random movements of the worms.
Chapter III. The influence of horizontal light of various intensities upon the worms.
Chapter IV. The influence of elevated light upon the worms.
Chapter V. The influence of flashlight upon the worms.
Chapter VI. The influence of light upon the worms with the brain removed.
Chapter VII. The axially graded reactions of the worms against light.
Chapter VIII. The influence of light upon regenerating worms.
Chapter IX. The phototactic antagonism between the brain and the ventral nerve cord. General conclusions.

Chapter I. The differential influence of light upon fresh and tired worms.

The object of the experiments was to ascertain whether or not under the influence of a constant light the same individual worm would show a constant reaction when it was tested repeatedly.

For the purpose of investigation an electric lamp of 500 watts was used as the light source and four experiments were carried on under the influence of a horizontal light at the distances of 1, 2, 3 and 4 meters respectively from the light source. In each experiment 30 worms were used and the same individual worm was used four times from the *first* to the *fourth trial.* In each instance the whole length of the body was illuminated (Parker and Arkin, 1901). Just before the trials, a 1% aqueous solution of methylene blue (Herms, 1911) was applied to each worm and the worm was put at right angles with the light rays; its body being straightened upon a white paper which had been previously well moistened (Parker and Parshley, 1911) and placed on a glass plate. Methylene blue was good enough to stamp precisely the tracks of the worms on the surface of the paper.

When I was working on Experiment I the reactions of the worms against the light appeared to me so similar in each individual that I was satisfied with the tests of only 30 individuals. The reason for limiting the number of trials to only four was that the same worm became gradually sluggish in motion at the fifth trial owing probably to fatigue and could not crawl out from the given position for a requisite distance.

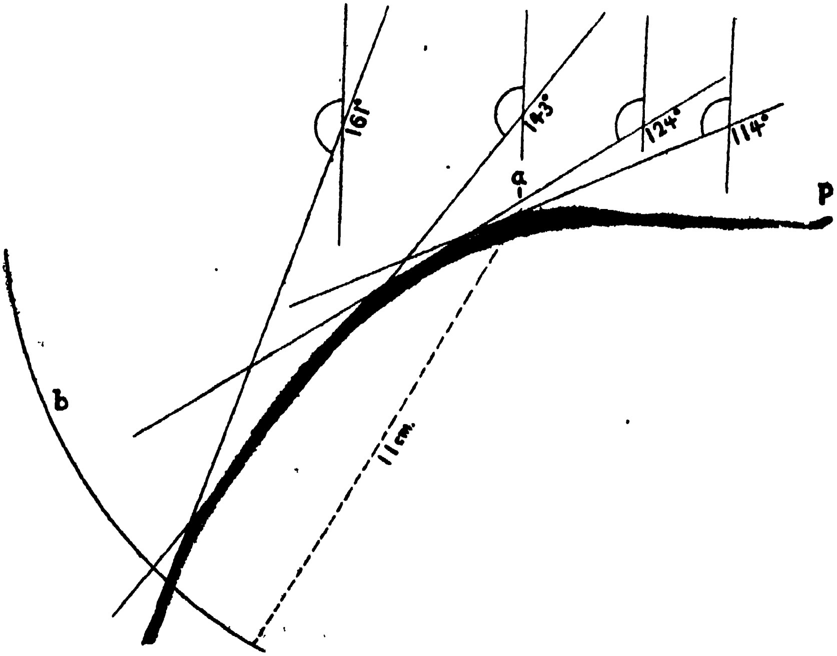
THE METHOD OF TREATING THE DATA.

Each track studied was limited within the circular area of 11 cm. radius, the center of which was located at the originating point of the crawl or the initial position of the anterior end of the body.

The state of the changes in the direction of the crawl is expressed quantitatively by four angles which were formed by the track of the movements of the anterior end of the body. It is to be stated that when measuring angles the rays from the light source were presumed always to be parallel. The angle between the direction of the rays and the given initial position is called the *given angle,* and in the present experiments it forms always 90°. The line of the rays and a tangent drawn at any given point of the track form two angles supplementary to each other and one angle which is farthest from the initial position of the worm is called the *occupied angle.* Finally the difference between the given angle and the occupied angle is called the *moved angle.* For example, put a worm at right angles to the light rays and let it crawl out and occupy a new position (say 135° to the rays), then in this case 90° is the magnitude of the given angle, 135° that of the occupied angle, and the difference between the two or 45° is that of the moved angle.

If we observe the crawling of a worm it does not progress continuously as for instance a snake does, but makes necessarily a short progress or step followed by a pause or stop. In summertime usually

FIG. 1.



A track from Table 1 (No. 9, First trial). 2/3 of natural size. The direction of light rays is represented by the longitudinal lines. *a*—starting point of crawl or the anterior end of the worm put at the given angle 90°, *b*—circular line which shows the border of the definite area, *P*—posterior end of the worm, 114°—first angle, 124°—second angle, 143°—third angle, 161°—fourth angle, 11 cm.—radius of the circle shown by *b*.

one progress measures about 1 cm. in distance without changing the direction and takes 3 or more seconds in time. The definitions of the four angles which are to express the changes in the direction of the crawl were derived from the above behavior of the worms. The occupied angle which was formed by the first progress starting from the given position, or by the turning movement [direct as well as indirect after a contraction of the anterior end of the body (Mast, 1911)] is called the *first angle*. Often a worm showed preliminary movements at the beginning of the crawl (Holmes, 1905 and Jennings, 1906), but the measurement of the first angle was made only after the worm described a definite track. The occupied angle which was formed by the second progress (or by the successive progresses if these made a continuous straight line) is called the *second angle*. The occupied angle near the border of the definite circular area is called the *fourth angle*. Finally the occupied angle which was formed by a straight line representing the best of the general outline of the track, which lies between the second and the fourth angles, is called the *third angle*. So the first, second and fourth angles are always real, while the third angle is sometimes real, sometimes imaginary, represented by a tangent or a straight line which expresses the probable course of the track.

As an actual example a complete track is shown in Fig. 1, in which the moving directions are expressed quantitatively by the following data:

1st angle	2nd angle	3rd angle	4th angle
114°	124°	143°	161°

The worm body of which was straightened was placed at right angles to the direction of the light rays. Before it crawled out a point *a* was marked at the anterior end of the body so that we may know afterwards from where it began to move. The worm started and made the first progress of about 1 cm. away from the light. This portion of the track is expressed by the first angle 114°. After a pause the second and a few successive progresses made the second angle 124°. The worm progressed for some distance tracing the curve which is approximately expressed by the third angle 143°, and finally it passed the marginal line of the definite area making the fourth angle 161°. So, as a whole, the track shows a case of negative phototaxis.

The occupied angles were studied statistically. The average of these is called the *average occupied angle*, and the difference between the average occupied angle and the given angle the *average moved angle*.

In the first column, Table 1, the first angles in the first trial are given. In this case 90° is the given angle, 122.47° the first average occupied angle, and consequently 32.47° the first average moved angle. However we notice that among 30 individuals Nos. 1—5 show positive phototaxis or smaller angles than 90°, while the remaining Nos. 6—30 show negative phototaxis or larger angles than the given angle, and thus the average value involves the worms whose phototaxes were opposite: that is positive and negative phototaxes. The occupied angle which expresses the degree of positive phototaxis is called the *positive occupied angle*, and that which expresses the degree of negative phototaxis the *negative occupied angle*. The difference between the given and the positive occupied angles is called the *positively moved angle* and that between the negative occupied and the given angles the *negatively moved angle*.

The positive and the negative occupied angles are obtained by the following formulae:

$$\text{Positive occupied angle} = \frac{\begin{array}{c}\text{The sum of the occupied angles}\\ \text{which show positive phototaxis}\end{array} + BC^{\circ}}{A+B}$$

$$\text{Negative occupied angle} = \frac{\begin{array}{c}\text{The sum of the occupied angles}\\ \text{which show negative phototaxis}\end{array} + AC^{\circ}}{A+B}$$

where A represents the number of positively phototactic individuals, B the number of negatively phototactic ones, and C° the magnitude of a given angle.

The average, positive and negative occupied angles are so related that either value can be obtained from the other two. In general: —

$$\begin{array}{c}\text{Positive}\\ \text{occupied angle}\end{array} = \begin{array}{c}\text{Average}\\ \text{occupied angle}\end{array} - \left(\begin{array}{c}\text{Negative}\\ \text{occupied angle}\end{array} - \begin{array}{c}\text{Given}\\ \text{angle}\end{array}\right)$$

$$= \begin{array}{c}\text{Average}\\ \text{occupied angle}\end{array} - \begin{array}{c}\text{Negatively}\\ \text{moved angle.}\end{array}$$

$$\begin{array}{c}\text{Negative}\\ \text{occupied angle}\end{array} = \begin{array}{c}\text{Average}\\ \text{occupied angle}\end{array} + \left(\begin{array}{c}\text{Given}\\ \text{angle}\end{array} - \begin{array}{c}\text{Positive}\\ \text{occupied angle}\end{array}\right)$$

$$= \begin{array}{c}\text{Average}\\ \text{occupied angle}\end{array} + \begin{array}{c}\text{Positively}\\ \text{moved angle.}\end{array}$$

$$\begin{array}{c}\text{Average}\\ \text{occupied angle}\end{array} = \begin{array}{c}\text{Negative}\\ \text{occupied angle}\end{array} - \left(\begin{array}{c}\text{Given}\\ \text{angle}\end{array} - \begin{array}{c}\text{Positive}\\ \text{occupied angle}\end{array}\right)$$

$$= \begin{array}{c}\text{Negative}\\ \text{occupied angle}\end{array} - \begin{array}{c}\text{Positively}\\ \text{moved angle.}\end{array}$$

The difference between the negative and the positive occupied angles or the sum of the positively and the negatively moved angles is called the *absolutely moved angle.* This is the average of the absolute values of the moved angles. In general: —

$$\begin{array}{c}\text{Absolutely}\\ \text{moved angle}\end{array} = \begin{array}{c}\text{Negative}\\ \text{occupied angle}\end{array} - \begin{array}{c}\text{Positive}\\ \text{occupied angle}\end{array}$$

$$= \begin{array}{c}\text{Negatively}\\ \text{moved angle}\end{array} + \begin{array}{c}\text{Positively}\\ \text{moved angle.}\end{array}$$

EXPERIMENTS AND THE DESCRIPTIONS OF THE RESULTS.

Experiment I. The phototaxis was studied by placing the worms at a distance of 1 meter from the light source, and the results are shown in Table 1.

Experiment II. The phototaxis was studied by placing the worms at a distance of 2 meters from the light source and the results are shown in Table 2.

Experiment III. The phototaxis was studied by placing the worms at a distance of 3 meters from the light source and the results are shown in Table 3.

Experiment IV. The phototaxis was studied by placing the worms at a distance of 4 meters from the light source and the results are shown in Table 4.

Experiments I, II and IV were carried on during the month of August, while Experiment III was carried on during the latter part of November.

In Tables 1–4 the data from each individual worm are given and are arranged according to the order of the magnitude of the first occupied angle in the first trial. Individual worms are numbered from 1 to 30, and the data on the second, third and fourth trials which belong to the same worm are given along corresponding horizontal lines.

If we examine each individual record in the tables it will be found

EXPERIMENT I.

TABLE 1.

Numbers of worms tested	First trials				Second trials				Third trials				Fourth trials			
	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles
	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees
1	58	76	52	35	45	45	55	113	77	90	129	161	90	138	146	164
2	60	67	50	0	100	79	52	27	66	90	38	0	75	53	23	5
3	62	77	55	41	113	113	90	66	65	79	52	41	90	90	69	52
4	82	82	115	124	84	69	56	56	114	88	75	75	56	77	66	56
5	86	61	38	18	85	90	57	24	90	53	58	37	86	75	54	28
6	91	91	95	85	106	68	59	49	61	76	71	62	90	59	74	53
7	92	109	142	157	91	122	90	102	110	76	92	84	72	75	72	72
8	106	98	120	127	113	77	87	87	91	91	72	72	93	75	84	65
9	114	124	143	161	82	82	109	153	90	79	87	90	79	102	92	69
10	117	146	174	160	59	66	120	141	118	102	90	100	99	125	112	95
11	118	93	59	38	83	83	49	14	77	60	43	23	105	105	45	9
12	122	138	168	180	109	147	123	110	98	103	112	112	90	90	81	100
13	123	92	48	20	93	93	81	68	108	95	74	58	90	77	63	39
14	124	176	158	180	116	116	128	114	74	93	93	93	90	95	0	24
15	126	110	132	167	92	92	80	80	65	82	99	99	79	89	65	65
16	127	113	142	162	110	103	111	89	134	107	89	76	106	101	77	74
17	132	113	138	166	69	82	91	97	90	82	96	92	83	71	57	57
18	133	155	149	180	90	90	93	104	79	88	90	84	73	83	69	69
19	141	166	180	142	90	90	101	125	92	114	150	120	92	92	106	106
20	141	180	151	180	92	122	104	113	143	162	127	106	93	112	87	75
21	142	117	163	180	165	172	174	180	180	180	180	180	161	152	180	180
22	142	161	180	164	128	150	142	168	94	94	115	115	127	114	135	102
23	145	114	167	156	84	135	124	96	93	110	116	116	90	75	95	95
24	146	124	153	180	137	119	110	121	95	102	98	98	71	85	83	77
25	147	167	166	180	162	180	166	180	102	120	150	150	74	80	94	114
26	149	180	168	180	177	163	154	172	93	117	147	161	90	153	127	94
27	156	127	164	180	111	142	165	161	95	122	146	125	108	116	116	116
28	158	122	165	180	118	90	104	120	88	98	112	118	76	54	75	69
29	158	177	169	164	153	153	166	180	133	123	107	129	109	100	106	116
30	176	174	180	180	106	123	146	180	119	126	144	157	92	99	94	118

EXPERIMENT II. TABLE 2.

Numbers of worms tested	First trials				Second trials				Third trials				Fourth trials			
	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles
	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees
1	59	68	68	68	105	127	152	180	61	61	84	71	53	28	28	28
2	74	123	173	178	95	95	85	66	102	90	62	48	60	75	64	52
3	90	60	28	0	79	64	34	34	90	116	107	117	90	90	83	83
4	90	99	115	122	99	99	124	108	52	73	45	63	99	90	95	95
5	90	105	93	105	64	88	94	101	122	105	99	114	128	101	92	85
6	90	106	90	62	106	150	110	140	90	58	39	28	90	66	46	30
7	90	141	133	142	141	102	110	119	116	99	105	114	74	74	64	64
8	94	109	170	164	90	90	117	121	112	112	127	127	123	144	130	139
9	96	96	51	15	100	86	81	81	121	121	145	180	75	75	85	93
10	98	42	53	53	156	130	178	161	83	156	125	114	88	132	148	129
11	99	105	117	134	83	100	146	180	78	85	72	81	90	77	115	134
12	103	96	93	91	116	90	115	115	103	103	138	115	96	83	75	81
13	107	90	112	103	71	71	47	35	68	68	56	56	86	98	90	94
14	109	109	79	54	76	87	58	34	90	42	86	104	65	65	88	111
15	109	109	91	91	138	138	125	133	83	72	58	51	83	96	90	90
16	115	150	180	155	140	101	105	112	130	96	121	121	118	153	124	124
17	117	117	77	59	80	64	47	26	86	75	66	49	75	74	70	70
18	120	140	107	68	72	57	43	34	90	63	36	23	65	65	34	32
19	120	146	162	162	127	114	123	139	72	90	78	78	98	80	64	64
20	129	106	114	114	144	89	67	37	138	138	95	83	136	136	111	111
21	131	159	151	158	138	127	115	115	107	150	118	85	90	100	98	98
22	134	164	155	180	149	129	150	131	133	155	132	156	152	134	148	136
23	147	147	147	147	111	111	87	87	96	96	103	90	64	64	77	68
24	151	120	72	40	84	77	46	22	90	76	65	53	63	86	66	66
25	154	165	180	117	82	75	78	67	85	77	88	88	83	83	72	54
26	154	173	180	180	129	154	172	180	87	87	69	42	92	92	102	90
27	156	167	145	136	90	90	90	90	88	83	75	68	90	106	113	118
28	164	152	180	180	90	69	59	47	174	142	132	117	129	150	116	116
29	171	171	180	180	121	90	110	110	90	77	44	36	92	92	73	55
30	176	176	180	180	90	90	120	108	116	132	155	180	67	92	106	86

EXPERIMENT III.

TABLE 3.

Numbers of worms tested	First trials				Second trials				Third trials				Fourth trials			
	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles
	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in dagrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees
1	60	60	52	48	135	117	110	110	89	89	100	100	96	62	92	92
2	68	104	148	171	127	149	180	163	113	168	167	159	110	163	177	178
3	69	57	57	57	143	123	142	142	113	98	108	124	116	146	169	169
4	75	65	31	4	90	70	66	66	88	88	108	101	90	90	105	117
5	85	107	131	131	109	140	144	154	101	88	103	110	99	90	83	83
6	90	116	124	136	104	59	78	78	95	123	100	100	109	129	96	87
7	90	121	152	180	62	62	75	75	78	78	84	79	103	103	108	118
8	97	97	103	112	60	75	82	82	90	95	99	90	61	85	77	70
9	102	102	98	98	150	150	129	129	118	95	103	103	85	88	88	88
10	106	160	180	162	96	96	69	35	50	50	73	41	101	140	116	100
11	107	119	110	101	147	138	147	162	106	70	46	46	100	121	132	132
12	107	163	167	156	156	163	173	180	90	116	147	173	123	131	155	172
13	110	110	157	170	144	117	128	149	119	143	171	153	120	166	128	109
14	128	114	130	117	129	157	164	179	99	90	118	108	79	84	80	72
15	128	116	151	180	123	152	135	180	104	76	120	120	112	112	103	96
16	132	112	105	93	128	101	113	126	92	92	97	97	75	37	55	55
17	136	180	124	169	82	77	72	88	90	78	71	71	83	89	76	76
18	140	140	170	180	116	94	100	116	90	154	135	156	124	124	104	98
19	140	169	163	168	132	154	164	164	139	180	158	139	146	146	125	108
20	141	101	134	134	168	144	153	167	162	140	129	115	131	111	97	93
21	141	170	160	174	142	142	103	103	147	180	160	180	48	83	90	85
22	142	171	149	172	119	108	146	180	119	145	165	180	114	150	141	141
23	144	161	175	175	83	83	83	83	90	81	85	78	94	105	86	110
24	155	167	177	171	149	149	172	163	90	75	63	67	64	105	128	114
25	159	130	176	180	165	168	176	171	112	163	140	140	127	124	179	128
26	166	124	102	85	117	117	108	88	81	57	44	22	79	79	69	63
27	166	176	158	180	87	87	69	44	116	90	81	81	143	113	107	89
28	167	170	178	171	125	152	145	179	114	129	120	133	102	133	155	127
29	177	177	177	177	111	135	118	152	90	109	109	95	67	85	104	119
30	180	175	161	161	117	109	118	103	90	132	104	93	128	104	97	97

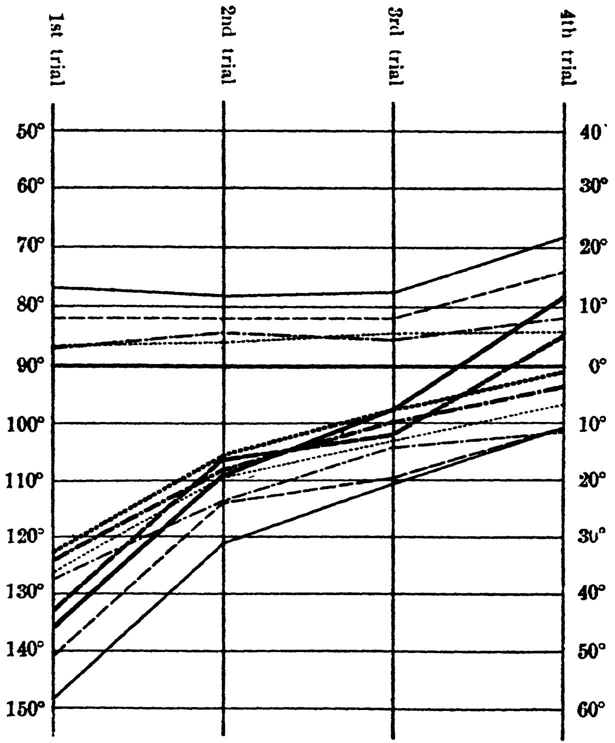
EXPERIMENT IV. TABLE 4.

Numbers of worms tested	First trials				Second trials				Third trials				Fourth trials			
	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles
	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees
1	47	70	44	44	69	13	45	28	8	42	56	65	118	45	116	52
2	51	58	36	1	72	83	47	2	80	55	22	0	81	62	46	46
3	65	65	45	51	105	72	57	39	63	63	51	67	110	131	124	144
4	71	91	141	176	113	146	132	107	93	93	128	109	119	94	136	136
5	72	82	70	70	94	94	89	104	123	111	119	128	89	83	90	90
6	73	89	85	99	74	74	57	39	88	88	75	65	83	83	79	66
7	74	81	136	167	87	77	72	88	95	127	147	147	97	97	90	90
8	74	95	73	52	29	29	16	0	163	137	175	146	90	78	57	40
9	79	74	67	56	87	129	119	131	86	86	90	102	101	95	124	123
10	83	83	70	47	107	107	103	90	100	100	97	113	85	77	62	49
11	86	75	61	45	118	95	41	18	90	85	67	37	85	85	89	94
12	90	76	41	15	112	125	77	52	90	139	105	79	76	99	61	59
13	90	80	65	65	63	96	89	74	90	97	139	115	90	120	138	114
14	93	73	92	92	112	93	137	178	90	72	112	87	55	104	73	73
15	93	107	84	71	117	100	135	135	122	114	114	114	122	93	106	106
16	102	72	102	79	92	92	76	92	90	99	106	112	79	90	80	90
17	102	90	63	75	97	97	102	95	135	145	172	143	72	81	90	90
18	105	128	116	89	116	94	79	61	87	125	77	45	63	85	72	79
19	107	107	109	124	84	79	66	50	145	96	120	105	125	145	104	86
20	109	148	171	159	102	102	90	90	90	123	67	57	65	65	76	62
21	113	136	131	147	113	78	100	126	83	72	88	80	96	96	105	115
22	114	85	52	19	92	117	166	180	100	123	107	107	90	90	143	143
23	116	81	39	0	92	112	68	31	124	174	149	180	115	96	54	8
24	123	159	141	154	124	180	136	165	107	90	81	65	90	60	76	63
25	125	135	121	149	151	117	104	109	72	79	90	100	84	77	83	83
26	132	92	78	85	77	77	55	71	94	94	85	85	90	90	54	36
27	144	105	146	180	98	98	68	49	112	158	122	85	94	94	110	126
28	147	170	153	177	84	84	105	125	114	114	132	158	90	85	101	110
29	157	171	180	180	101	101	81	62	105	105	65	44	90	90	68	54
30	171	180	178	159	90	90	53	33	91	91	47	38	62	90	39	14

EXPERIMENT I. TABLE 5.

		1st angles	2nd angles	3rd angles	4th angles
		in degrees	in degrees	in degrees	in degrees
Average occupied angles	1st trial	122.47	124.33	132.80	135.57
	2nd trial	105.43	108.53	106.23	109.67
	3rd trial	97.80	100.07	101.73	97.80
	4th trial	90.97	93.73	84.90	78.60
Positive occupied angles	1st trial	86.60	87.10	82.07	76.90
	2nd trial	85.70	84.70	82.20	78.68
	3rd trial	84.73	85.43	81.97	77.40
	4th trial	84.47	82.27	74.13	67.93
Negative occupied angles	1st trial	125.87	127.23	140.73	148.67
	2nd trial	109.73	113.83	114.03	120.99
	3rd trial	103.07	104.64	109.76	110.40
	4th trial	96.50	101.46	100.77	100.67

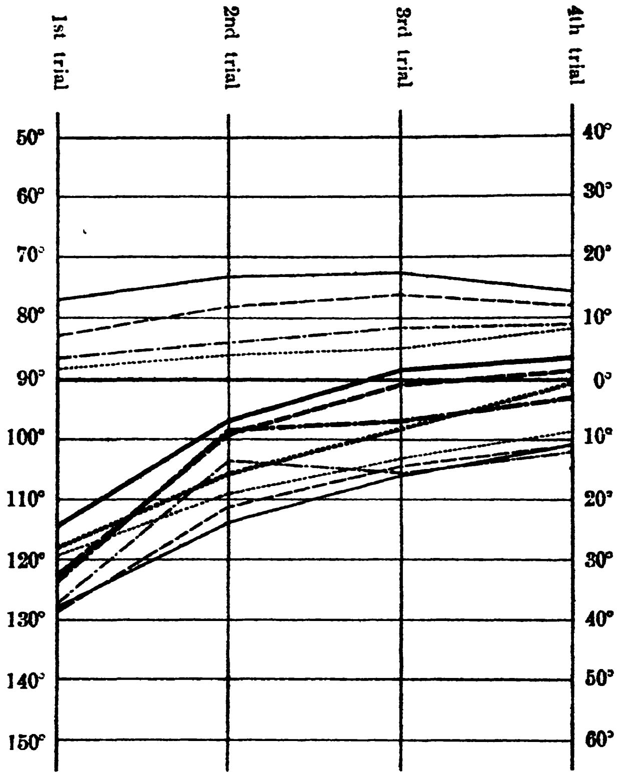
EXPERIMENT I. FIG. 2.



EXPERIMENT II. TABLE 6.

		1st angles	2nd angles	3rd angles	4th angles
		in degrees	in degrees	in degrees	in degrees
Average occupied angles	1st trial	117,90	123.70	122.53	114.60
	2nd trial	105.53	98.47	99.60	97.10
	3rd trial	98.43	96.77	90.83	88.40
	4th trial	90.63	93.37	88.83	86.53
Positive occupied angles	1st trial	88.43	86.67	83.27	76.97
	2nd trial	86.03	84.57	78.40	73.00
	3rd trial	85.10	81.40	76.10	72.43
	4th trial	81.53	81.17	77.97	75.60
Negative occupied angles	1st trial	119.47	127.03	129.26	127.63
	2nd trial	109.50	103,90	111.20	114.10
	3rd trial	103.33	105.37	104.73	105.97
	4th trial	99.10	102.20	100.86	100.93

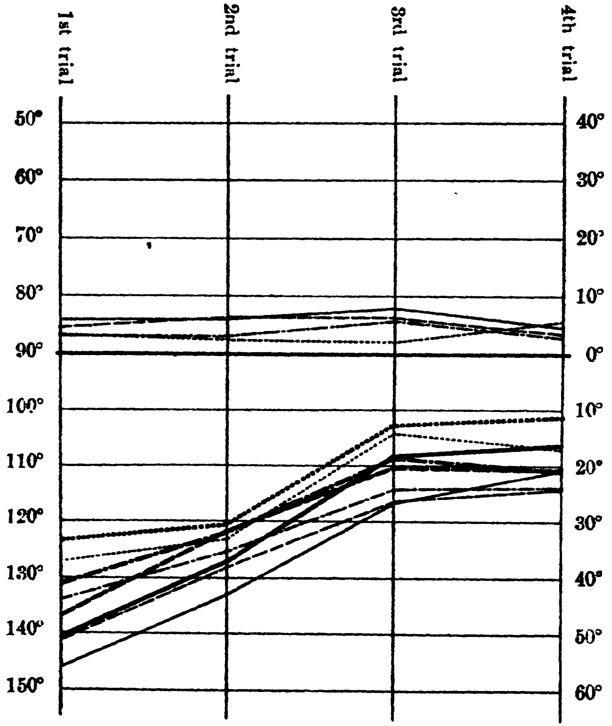
EXPERIMENT II. FIG. 3.



EXPERIMENT III. TABLE 7.

		1st angles	2nd angles	3rd angles	4th angles
		in degrees	in degrees	in degrees	in degrees
Average occupied angles	1st trial	123.60	131.13	136.67	140.43
	2nd trial	120.53	122.27	122.07	127.20
	3rd trial	102.50	109.07	110.27	108.47
	4th trial	100.97	110.93	110.73	106.20
Positive occupied angles	1st trial	86.90	87.07	85.67	84.63
	2nd trial	87.47	87.10	84.13	84.30
	3rd trial	87.87	84.67	84.23	82.17
	4th trial	84.37	87.00	86.47	85.60
Negative occupied angles	1st trial	126.70	134.06	141.00	145.80
	2nd trial	123.06	125.17	127.94	132.90
	3rd trial	104.63	114.40	116.04	116.30
	4th trial	106.60	113.93	114.26	110.60

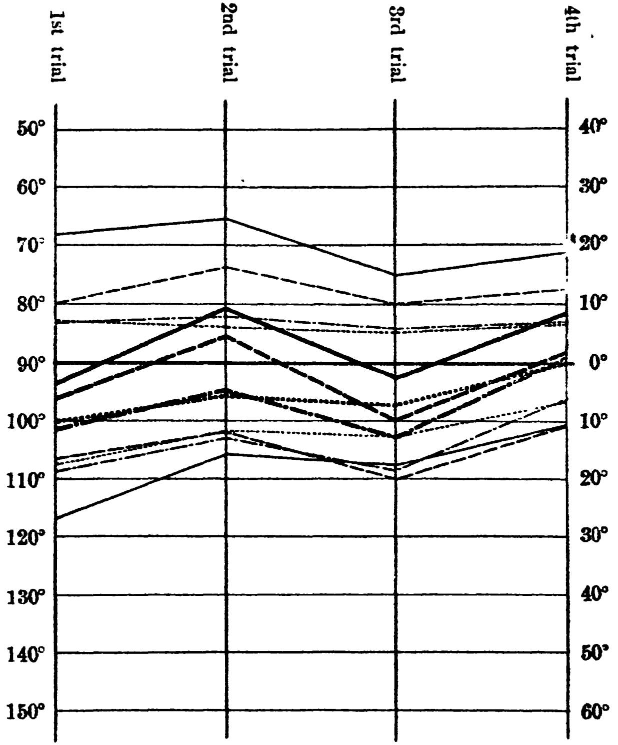
EXPERIMENT III. FIG. 4.



EXPERIMENT IV. TABLE 8.

		1st angles	2nd angles	3rd angles	4th angles
		in degrees	in degrees	in degrees	in degrees
Average occupied angles	1st trial	100.27	101.93	96.33	94.23
	2nd trial	95.73	95.03	85.50	80.80
	3rd trial	97.67	103.23	100.17	92.60
	4th trial	90.20	89.33	88.20	81.37
Positive occupied angles	1st trial	82.83	83.13	79.91	67.80
	2nd trial	84.20	82.20	73.87	65.30
	3rd trial	84.90	84.40	80.03	74.97
	4th trial	83.63	83.20	77.63	71.00
Negative occupied angles	1st trial	107.44	108.80	106.42	116.43
	2nd trial	101.53	102.83	101.63	105.50
	3rd trial	102.77	108.83	110.14	107.63
	4th trial	96.57	96.13	100.57	100.37

EXPERIMENT IV. FIG. 5.



that in Nos. 18 and 24 in Table 1 the magnitude of the corresponding angles is gradually decreased in the successive trials. Similar phenomena are found in Nos. 7, 8, 9, 11, 12, 14, 15, 16, 17, 19, 20, 22, 25, 26, 27, 28, 29, 30 in Table 1, Nos. 2, 7, 18, 19, 21, 23, 25, 29 in Table 2, Nos. 15, 17, 19, 25, 28, 30 in Table 3 and Nos. 18, 20, 24, 25, 29, 30 in Table 4, though in some worms more or less irregularities are found. This fact shows that in these worms just mentioned when the trial is repeated the negative phototaxis at the beginning tends to become positive phototaxis. On the other hand, however, some worms show an utterly irregular phototaxis especially in the experiments which were carried on under the influence of a light of comparatively low intensity.

From the average, positive and negative occupied angles which were calculated from Tables 1–4, Tables 5–8 respectively were made and the data were plotted in Figs. 2–5.

In Figs. 2–5, the trials are shown on abscissa making an equal distance from one to the other successively and the angles are scaled on ordinate. The scale at the left side is of the occupied angles and that at the right side is of the moved angles. The latter scale originates at the given angle 90°, and the one going upwards expresses the positively moved angle and the other going downwards expresses the negatively moved angle. The first angles are denoted by the dotted line, the second angles by the chain line, the third angles by the broken line and the fourth angles by the full line. The average angles are denoted by the thick lines, and the positive and negative angles by the fine lines. In these figures the state of changes in the magnitude of the absolutely moved angles in accordance with the progress of the trial is represented by the interspace between the tracings of the corresponding positive and negative occupied angles. For instance, the interspace between the fine full lines represents the state of changes in the fourth absolutely moved angles.

Let us study in Tables 5–8 and Figs. 2–5 the state of changes in the angles in accordance with the progress of the trial. In Experiments I, II and III the average and the negative occupied angles show in general a regular decrease from the first to the fourth trial. The magnitudes of the positive occupied angles in Experiment I are nearly the same in the first, second and third trials, but decrease more or less suddenly in the fourth trial while those in Experiments II and III decrease gradually from the first to the third and tend to increase in the fourth. In Experiment IV the state of changes is quite different in comparison with the cases of the former three experiments and is utterly irregular numerically as is seen in Table 8, but in Fig. 5 it is seen very roughly that the positive occupied angles tend to increase from the second to the fourth trial while the negative occupied angles tend to decrease from the first to the fourth, both tracings approaching gradually to the abscissa passing the given angle 90° and the corresponding positively and negatively moved angles make nearly an equal magnitude in each trial and consequently the magnitude of the average occupied angles approaches nearly to that of the given angle.

The absolutely moved angles in each experiment show generally a regular decrease of the magnitude in accordance with the progress of the trial, though some irregularities are found.

Since in Experiment I, in which the worms were exposed to a strong light, the state of changes in the angles or in the degrees of phototaxis of the worms is regular and more distinct than in Experiments II and IV which were carried on in the same season as Experiment I and in which gradually decreasing intensities of light were used, we may state that the degree of irregularities of reaction is inversely proportional to the intensity of the light. The discordantly strong, negative phototaxis shown in Experiment III is probably due to the difference of the season in which the worms were tested. Moreover it is worthy of note that in Experiments I and II the difference between the corresponding negative occupied angles in the successive trials is generally larger between those in the first and second trials than between those in the remaining ones. The same tendency is seen also in Experiments III and IV, though there are many irregularities.

Thus far we have shown the changes of tracks expressed in terms of the magnitude of the angles. It seems however also important to determine more directly the frequency distribution of the worms in the circular area at the end of each trial, in order to see how many worms crawled toward the light and how many crawled away from it taking 90° as the starting line. Such data will not only clarify the

TABLE 9.

Angular sections		0°-22°		23°-67°		68°-112°		113°-157°		158°-180°	
		%	No.	%	No.	%	No.	%	No.	%	No.
Experiment I.	1st trial	10	3	10	3	3	1	17	5	60	18
	2nd trial	3	1	17	5	30	9	27	8	23	7
	3rd trial	3	1	17	5	43	13	27	8	10	3
	4th trial	7	2	30	9	43	13	13	4	7	2
Experiment II.	1st trial	7	2	17	5	20	6	27	8	30	9
	2nd trial	3	1	30	9	27	8	27	8	13	4
	3rd trial	0	0	33	10	30	9	30	9	7	2
	4th trial	0	0	30	9	47	14	24	7	0	0
Experiment III.	1st trial	3	1	7	2	17	5	17	5	57	17
	2nd trial	0	0	10	3	30	9	23	7	37	11
	3rd trial	3	1	10	3	47	14	27	8	13	4
	4th trial	0	0	7	2	57	17	27	8	10	3
Experiment IV.	1st trial	13	4	23	7	27	8	13	4	23	7
	2nd trial	10	3	33	10	33	10	13	4	10	3
	3rd trial	3	1	30	9	37	11	23	7	7	2
	4th trial	7	2	33	10	37	11	23	7	0	0

former statements of the angular variation, but still other additional physiological factors might be revealed. The results of the determinations are given in Table 9. In the table the angle from 0° to 180° is divided into 5 sections, namely 0°-22°, 23°-67°, 68°-112°, 113°-157° and 158°-180° and the number of the individuals who crawled in each section was determined and the values are given both in percentage and in absolute numbers. We notice at once in the table that in accordance with the progress of the trial the number of individuals decreases in the section 158°-180° while it increases in the section 68°-112°. In the section 0°-22° the state of the distribution shows rather a tendency to decrease the number of individuals from the first to the fourth trial though there are some irregularities, and it is common too to each experiment that in the fourth trial the number of individuals is maximum in the section 68°-112° while in the first trial it is in the section 158°-180°. These facts appear to me to show that when the worms are fatigued owing to repeated trials the phototaxis becomes indistinct and consequently fails to exhibit a strong phototaxis, both positive and negative, making very slight alteration from the given angle 90°.

As will be seen in the fundamental tables (Tables 1-4) some worms showed the tendency to positively phototactic movements in all experiments. It will therefore be interesting to examine more in detail the movements of the positively phototactic worms as the trials progress. The number of individuals which crawled toward the light or showed occupied angles from 0° to 89° is shown in Table 10 both in percentage and in absolute numbers. In Experiment I the number of individuals at each angle increases in accordance with the progress of the trial and nearly similar change is seen in Experiments II and III, while in Experiment IV the change is utterly irregular. It is also recognizable in the table that in Experiment I the percentage values at the third and fourth angles in the fourth trial amount to more than half, while at the second, third and fourth angles in the third trial and at all the angles in the fourth trial in Experiment II, and at the second, third and fourth angles of the first trial, at the third and fourth angles in the second and third trials and at all the angles of the fourth trial in Experiment IV the percentage value is nearly half. The smaller percentage value in Experiment III in comparison with the other experiments is probably due to the strong, negative phototaxis of winter specimens as already stated.

I therefore conclude from the above that when the worms are exposed to a stronger light the majority of them may become positively phototactic at the fourth trial, but when the worms are exposed to a weaker light half of them exhibit the positive and half of them the negative phototaxis all through the trials.

We also notice in the fundamental tables some worms decrease the magnitude of the occupied angles gradually from the first to the fourth trial; some to the third and some to only the second. These

TABLE 10.

		1st angles		2nd angles		3rd angles		4th angles	
		%	No.	%	No.	%	No.	%	No.
Experiment I.	1st trial	17	5	17	5	20	6	23	7
	2nd trial	27	8	30	9	30	9	33	10
	3rd trial	30	9	33	10	33	10	37	11
	4th trial	37	11	47	14	60	18	60	18
Experiment II.	1st trial	7	2	10	3	23	7	30	9
	2nd trial	30	9	37	11	43	13	40	12
	3rd trial	37	11	47	14	53	16	50	15
	4th trial	47	14	47	14	50	15	50	15
Experiment III.	1st trial	17	5	10	3	10	3	13	4
	2nd trial	17	5	23	7	27	8	30	9
	3rd trial	17	5	37	11	27	8	27	8
	4th trial	30	9	27	8	27	8	33	10
Experiment IV.	1st trial	37	11	50	15	53	16	57	17
	2nd trial	33	10	33	10	60	18	53	16
	3rd trial	27	8	30	9	40	12	50	15
	4th trial	43	13	43	13	53	16	53	16

movements appear to be related in an interesting and suggestive manner to the intensity of the light. For the purpose of determination I have enumerated such instances in Table 11. In the table the number of individuals which exhibited such movement are shown both in percentage and in absolute numbers; that is the worms in whose movement the occupied angles decreased from the first to the fourth trial are denoted by the formula $I > II > III > IV$, those which showed decrements up to the third by $I > II > III < IV$, and those up to the second by $I > II < III \gtrless IV$. The table shows that (1) the number of individuals decreases in accordance with the increase of the distance from the light source or with the decrease of the intensity of the light, and that (2) the number of individuals which showed the decreased magnitude of angles for 2 successive trials is larger than those for 3 trials and the latter are larger than those for 4 trials. It is also to be noted that in Experiments III and IV where the influence of the lower intensity of light was studied the number of individuals which showed the decreased magnitude of angles for only 2 successive trials hardly surpassed 50% of the entire number of cases examined.

TABLE 11.

	I>II<III≷IV		I>II>III<IV		I>II>III>IV	
	%	No.	%	No.	%	No.
Experiment I.	80	24	70	21	67	20
Experiment II.	78	22	40	12	27	8
Experiment III.	57	17	40	12	20	6
Experiment IV.	57	17	27	8	20	6

It thus follows from the above that so far as the present experiments go the stronger light gives the worms a tendency to continue the decrease of the magnitude of the angles and *vice versa.*

GENERAL REMARKS.

As a convenience I wish to summarize the statements made in the proceeding pages.

As was noted before, the magnitude of the average angles decreases gradually according to the progress of the trial in each of Experiments I, II and III. However the number of individuals which behaved in the manner just stated is about 27% in Experiment II and 20% in Experiment III, while in Experiment I it is as high as 67% (Table 11). The discrepancies just noted make a definite conclusion difficult and we must seek for a satisfactory interpretation in some other factors. For the purpose of discussing such factors as just mentioned we wish to study the changes in the negative and positive occupied angles.

In Tables 5, 6 and 7 it will be seen that the negative occupied angles tend to decrease in accordance with the progress of the trial, and nearly the same change appears even in Table 8. The data given in Table 9 show decisively a similar reaction. From the definition the magnitude of the negative occupied angles can never be smaller than 90° and at the same time the difference between the successive angles is the largest in the first 2 successive trials and diminishes gradually in the following successive trials. If we combine these facts it follows that the tracings of the corresponding negative occupied angles can not be straight lines, but curves which resemble hyperbola.

The changes in the positive occupied angles are too complicated to explain briefly. In Table 5 (Experiment I) the magnitude of the positive occupied angles is minimum in the fourth trial which of course means that the reaction is strongly positive phototactic. This fact is clearly brought out in Table 10 (Fourth trial, Experiment I) where the number of individuals is maximum. In Tables 6 and 7 (Experiments II and III) the magnitude of the positive occupied angles is minimum in the third trial while the number of individuals in Table 10 is nearly maximum in both the third and fourth trials. Furthermore in Table 8 (Experiment IV) we notice that the minimum magnitude of the positive occupied angles is found in the second trial, yet the number of individuals in Table 10 is nearly equal in each trial. In the above the fact that the number of individuals is nearly constant in successive trials, while the magnitude of the positive occupied angles increases, means that the magnitude of the positively moved angles decreases in each worm or the positive phototaxis of each worm gradually becomes obscure. So the above statement should mean that in the majority of the worms the gradual decrease of the magnitude of the angles ceases early at the third trial in Experiment IV and at the fourth trial in Experiments II and III, while in Experiment I such decrement of the angles occurs from the first trial and continues throughout all subsequent trials. While I was carrying on Experiment I, it was noted that some individuals, though they previously made the regular change of tracks yet when the fifth trial was made, showed an increase of the magnitude of the angles and that in one case after a long rest the angle increased almost to 180° indicating an extremely strong, negative phototaxis. We may infer from the above fact just stated that even in Experiment I the decrease of the magnitude of the angles may not continue longer, but on the contrary even an increase may take place after a certain number of trials. So my conclusion indicates that the magnitude of the positive occupied angles may increase at the fifth or sixth trial in Experiment I, at the third or fourth trial in Experiments II and III, and at the third trial in Experiment IV, and may become irregular later.

According to the above statements the magnitude of the negative occupied angles decreases in accordance with the progress of the trial and approaches gradually to the given angle, while the magnitude of the positive occupied angles decreases first and then tends to increase thus approaching the given angle. Here it must be recalled that the magnitude of the absolutely moved angles also tend to decrease in accordance with the progress of the trial. Then the changes in the average occupied angles are chiefly influenced by the greater variation of the positive occupied angles: that is in compliance with the increase of the distance from the light source or with the decrease of the intensity of the light the magnitude of the average occupied angles decreases during a past of the trial and then tends to approach the given angle.

CONCLUSIONS.

1. If an individual worm is tested repeatedly the general feature of the track differs in each trial (See Tables 1–4).

2. Under the influence of the stronger intensity of the light the state of the changes in the tracks is generally regular either when tested once or repeatedly, and the more the intensity decreases the more the irregularities increase.

3. If the worms are tested repeatedly under the influence of the higher intensity of the light the majority change from the negative phototaxis to the positive, as will be seen from the gradual decreasing of the magnitude of the occupied angles which is due probably to

fatigue, while under the influence of the lower intensity of the light the worms make from the beginning free and irregular movements owing probably to a less fatigued condition.

Chapter II. The average limit of the free or random movements of the worms.

The primary object of the experiments here was to determine how the worms would change the direction of crawl when they were released after being exposed to light.

For the purpose of investigation a 500 watts electric lamp, as the light source, was put in an iron box, on one side of which there was a hole. An iron plate was made to slide up and down over the hole, so that when it was lifted up the light rays would pass through the hole and illuminate each worm laterally, but when it was put down it would shut off the light quickly.

After the treatment with methylene blue just as in Chapter I, the worms were at first exposed to the light at a distance of **2** meters from the light source, the intensity of which was equivalent to about 100 meter-candles, and then the light was shut off quickly. The darkness in the experimenting room was such that when the eyes were trained the outlines of the white paper could be dimly seen. The various angles which determine the track of the worm were determined in the same way as in Chapter I taking the electric lamp as a fixed position.

The worms were placed at different given angles against the light source; that is at 0°, 45°, 90°, 135° and 180°; 0° means that the anterior end of the worm was faced toward the light. The effect of the light at each given angle was determined from the study of 100 fresh worms, which were tested individually. Half of the worms were tested in the middle part of June and the remaining half in the latter part of July, However it was found that the results from both experiments were nearly the same, so these two data were treated as one.

In order to study how the worms crawled forward from the given angle, or from the given direction, the frequency distribution of the worms at any given angle was determined. It is evident that wherever the worms move round they necessarily occupy the area which can be expressed by the angles from 0° to 180°. For this reason the field of movements was divided into 5 angular sections, namely 0°–22°, 23°–67°, 68°–112°, 113°–157° and 158°–180° and the number of individuals found in each section is given in Table 12 expressed in

TABLE 12.

Given angles		0°–22°	23°–67°	68°–112°	113°–157°	158°–180°
0°	1st angles	64	29	7	0	0
	2nd angles	62	33	4	1	0
	3rd angles	54	40	6	0	0
	4th angles	51	40	9	0	0
45°	1st angles	17	70	13	0	0
	2nd angles	12	81	6	1	0
	3rd angles	19	69	12	0	0
	4th angles	18	62	19	0	1
90°	1st angles	2	20	52	25	1
	2nd angles	1	15	68	15	1
	3rd angles	1	21	61	17	0
	4th angles	4	19	52	23	2
135°	1st angles	0	0	15	70	15
	2nd angles	0	0	11	77	12
	3rd angles	0	0	16	64	20
	4th angles	0	1	18	62	19
180°	1st angles	0	0	3	37	60
	2nd angles	0	0	1	20	79
	3rd angles	0	0	1	42	56
	4th angles	0	0	2	42	56

percentage value. The sections which give the maximum frequency are called conveniently the maximum sections. The following 3 facts may be seen in the table.

1. At the given angle 0° the majority of the worms move slightly from the given direction (0°-22°). Similarly at the given angle 45° the majority of the worms remain at 45° or move slightly from the given direction (23°-67°). The same rule seems to hold also at the given angles 90°, 135° and 180°. In general, the majority of the worms tend to remain at any given angle and the frequency tends to be smaller in both directions from the maximum section.

2. At the given angle 45° the number of individuals found in the section 0°-22° is nearly equal to the sum of the worms found in the sections 68°-112°, 113°-157° and 158°-180°, at the given angle 90' the sum of the worms in the sections 0°-22° and 23°-67° to that in the sections 113°-157° and 158°-180°, and at the given angle 135° the sum of the worms in the sections 0°-22°, 23°-67° and 68°-112° to the number of the worms in the section 158°-180°. At the given angles 0° and 180° the number of individuals on both sides of the given position is assumed to be equal. So all the above relations ought merely to mean that the probability of movements of the worms from the given position is the same on both sides, left and right.

3. In the maximum section the second angle gives the maximum number of individuals in 4 cases out of 5, while the second angles in both sides of the maximum section give the minimum number. This means that the comparatively large, first moved angle was decreased at the second moved angle as the worms turn toward the given original direction. A similar behavior of the movement appears even in a lighted room; that is to say many worms show a strong tendency of change of direction either toward or away from light at the first progress which is soon followed by restoration of the given original direction at the second progress. A similar statement usually applies to those worms which were placed at 0°.

In Table 13, the occupied angles are arranged on the left side and the moved angles on the right. The position of the electric lamp was taken as the fixed position and the angles which moved away from this fixed position are denoted by - sign and the others represent the angles which moved toward the fixed position. (It is to be noted here

TABLE 13.

	Given angles	Occupied angles				Moved angles			
		1st angles	2nd angles	3rd angles	4th angles	1st angles	2nd angles	3rd angles	4th angles
		in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.
Average angles	0°	22.99	22.87	25.61	28.24	-22.99	-22.87	-25.61	-28.24
	45°	45.93	42.37	42.73	44.86	-0.93	2.63	2.27	0.14
	90°	91.89	88.89	87.99	89.58	-1.89	1.11	2.01	0.42
	135°	135.55	134.59	134.96	133.82	-0.55	0.41	0.04	1.18
	180°	159.52	166.43	160.78	158.73	20.48	13.57	19.22	21.27
Positive angles	0°	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	45°	37.86	37.59	35.23	33.97	7.14	7.41	9.77	11.03
	90°	80.49	80.72	78.03	76.89	9.51	9.28	11.97	13.11
	135°	127.91	129.23	124.90	123.83	7.09	5.77	10.10	11.17
	180°	159.52	166.43	160.78	158.73	20.48	13.57	19.22	21.27
Negative angles	0°	22.99	22.87	25.61	28.24	-22.99	-22.87	-25.61	-28.24
	45°	53.07	49.78	52.50	55.89	-8.07	-4.75	-7.50	-10.89
	90°	101.40	98.17	99.96	102.69	-11.40	-8.17	-9.96	-12.69
	135°	142.64	140.36	145.06	144.99	-7.64	-5.36	-10.06	-9.99
	180°	180.00	180.00	180.00	180.00	0.00	0.00	0.00	0.00

that the formulae on pages 297 and 298 are in regard only to the absolute values of the angles). When the worms were put at the given angle 0°, the positive occupied angles must be equal to 0° and consequently the average occupied angles must coincide with the negative occupied angles. At the given angle 180° for the same reason the negative occupied angles must be equal to 180° and consequently the average occupied angles must coincide with the positive occupied angles.

From Table 13, Fig. 6 was plotted. In this figure the first to the fourth angles are shown in abscissa and conveniently are plotted in an equal distance from one another and the magnitude of the occupied angles in ordinate. Each tracing of the average occupied angles is denoted by the full line and those of the positive (upper) and negative (lower) occupied angles by the broken lines, taking the value of the given angle as the starting point. The interspace between the tracing of the positive occupied angles and the abscissa passing the given angle expresses the positively moved angles, the interspace between the abscissa and the tracing of the negative occupied angles the negatively moved angles and consequently the interspace between the corresponding positive and negative occupied angles the absolutely moved angles. At the given angles 0° and 180° the interspace between the abscissa and the tracing of the average occupied angles expresses directly the absolutely moved angles. Since at the given angles 0° and 180° the average moved angles are identical with the absolutely moved angles and hence they must be equivalent in nature with the sum of the positively and negatively moved angles in comparison with the cases at the other given angles, each average moved angle at 0° is therefore comparable with twice the corresponding negatively moved angle at other given angles, and similarly at 180° with twice the corresponding positively moved angle at other given angles.

The following facts may be seen in Table 13 and Fig. 6.

1. At the given angles 45°, 90° and 135° the magnitude of the positively moved angle is nearly equal to that of each corresponding negatively moved angle. This fact shows that in a dark place the worms can crawl equally toward either the left or the right side from the given direction. The same movement should occur unquestionably at the given angles 0° and 180° and therefore the fact coincides with the second fact given in Table 12.

2. At the given angles 45°, 90° and 135° the magnitude of the average occupied angles are nearly equal to the magnitude of the given angles respectively. The same relation should be shown at the given angles 0° and 180°, if we assume that the worms made nearly the same amount of lateral deviation which is negative at 0° and positive at 180° and the averages of respective values are taken.

3. In spite of the different magnitude of given angles the state of changes in the absolutely moved angles is nearly the same (See

FIG. 6.

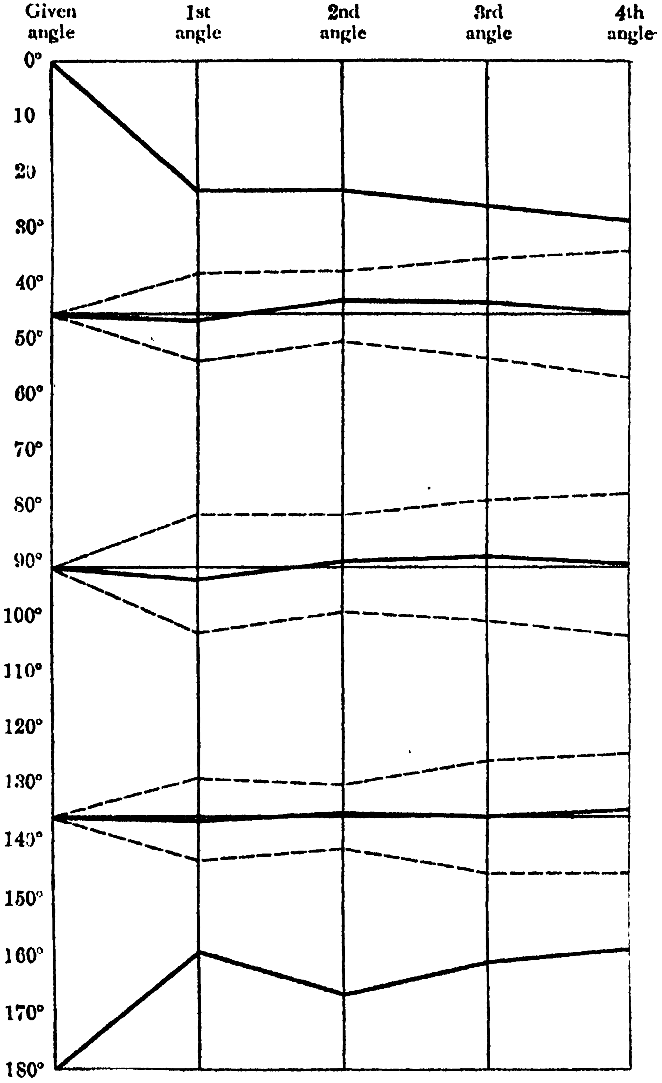


Fig. 6). This fact shows that even though the worms were placed at various positions, when they started to crawl in the dark the amount of the deviation from the given direction is nearly equal.

showing that the influence of the initial illumination has very little effect.

4. At each given angle the magnitude of the absolutely moved angle is decreased at the second and then is increased at the third and fourth. The same change is seen in both positively and negatively moved angles at the corresponding given angles 45°, 90° and 135°. This fact coincides with the third fact given in Table 12.

5. The positively and negatively moved angles show a deviation from the given direction in a dark place after the worms were influenced by the horizontal light. The average deviation at the first angle is ±9.43°, that at the second angle +7.72°, that at the third angle ±10.42° and that at the fourth angle ±11.84°. For the purpose of comparison I have carried on another experiment in which the light was placed right overhead (Elevation angle 90°, Chapter IV). The object of the experiment was to determine how the worms will crawl when allowed to crawl from the beginning in either a dark room or a light room under the isodynamic influence of light. Altogether 75 worms were tested and the following results were obtained: ±5.53° at the first angle, +5.97° at the second angle, ±8.73° at the third angle and ±12.51° at the fourth angle. We thus notice that the first angle of deviation in the experiment which was carried on under the influence of the horizontal light is larger nearly ±4.00° on the average than that in the experiment which was carried on under the overhanging light. The difference may be due to the effect of the horizontal light at the beginning of the tests, the intensity of which is nearly 100 meter-candles. The data appear also to show that the effect produced by the horizontal light at the first angle ought to influence the subsequent movement of the worms. But such influence is small and does not surpass 2.00° in angular value. So the movement of the worms after the second progress may be considered as a free or random movements in either experiment. The fourth angle of deviation from the former experiment is ±11.84° and that from the latter experiment is ±12.51°. The result show the near identity of the magnitude of deviation, and judging from this close similarity the deviation in the second and third angles which amounted to 2.00° may be considered as due to the difference in nature of the experiments in two cases; that is in the case where the worms were illuminated from above it produces a relatively smaller change in the second

and third angles compared with the case of the horizontal light, but at the fourth angle both give random movements thus producing greater similarity in the values or in round figure ±12°.

In the present experiment the worms must be influenced also by the radiating heat from the light source. Naturally all data in Tables 12 and 13 are directly applicable to the cases of the thermotaxis of the worms, and the data reveal that the thermotaxis was not pronounced in such a slight difference of temperature as in this experiment, thought according to Smith's experiments (1902) the negative thermotaxis of the worms is nearly decisive against either the higher or the lower temperature than the ordinary.

CONCLUSIONS.

1. When the worms started to crawl in the dark place, after being stimulated by the horizontal light, the difference of the given angles gives very slight effect.
2. Each worm can deviate equally toward either the left or the right side of the given position and if it shows a strong deviation at the first progress, then at the second progress it restores the initially given direction.
3. At least at the first progress of the crawl, the worms are influenced by the removed horizontal light.
4. The average deviation in the random movements of the worms is nearly ±12.00° at the fourth angle or at the distance of 11 cm. from the starting point.
5. The thermotaxis of the worms was not pronounced in the present experiment.

Chapter III. The influence of horizontal light of various intensities upon the worms.

Harper (1905), working on Perichaeta bermudensis and a species of Lumbricus, concluded that in light of comparatively low intensity worms make several random movements, but in direct sunlight, especially if the worms have previously been kept in darkness, random movements are almost entirely eliminated, while Adams (1903) says that "Allolobophora foetida is positively phototropic toward an electric incandescent lamp of 0.0011 candle-meter intensity." In my work in Experiments I, II and IV in Chapter I, it was indicated that the negative phototaxis gradually is pronounced with the increasing intensity of light. The object of the present experiments was to ascertain the fact just mentioned more in detail and to know if possible the true phototactic movements of the worms.

For the purpose of investigation an electric lamp of 500 watts was used as the light source and four experiments were carried on under the influence of the horizontal light at each distance of 1, 2, 3 and 4 meters from the light source. In each experiment a number of the worms were put at the given angles 0°, 45°, 90°, 135° and 180°. Each worm was brought out newly from a reservoir and was treated with methylene blue as in Chapter I just before the trial.

The measurement of the angles was at first made in the same way as in Chapter I, but since the result from each angle in tracks has shown the same tendency of conclusions, in the present chapter the result from the fourth angle alone is stated to avoid the repetition of the same statements.

Experiment I was carried on at a distance of 1 meter from the light source in the earlier part of August. Altogether 250 worms (50 worms at each given angle) were used in the experiment.

Experiment II was carried on at a distance of 2 meters from the light source. 100 worms were used at each given angle, the half of which were used in the latter part of June and the remaining half in the middle of July. Since the results obtained from both experiments were nearly identical they were united into one.

Experiment III was carried on at a distance of 3 meters from the light source in the latter part of July. 50 worms were used at each given angle.

Experiment IV was carried on at a distance of 4 meters from the light source in the earlier part of August. 50 worms were used at each given angle.

For the purpose of comparison the data from the above experiments and the data which were given in Chapter II were gathered together in Tables 14 and 15.

In Table 14 the frequency distribution of the worms is shown in percentage value at each given angle and at each distance from the light source. The table consists of two parts, one the distribution of worms and the other the phototaxis. In the part of the distribution of worms the field was conveniently divided into 4 angular sections, viz. 0°-45°, 46°-90°, 91°-135° and 136°-180°. In the part of the phototaxis those worms which showed similar phototaxis was gathered. To judge the phototaxis of each worm, the principle which was given on "the average limit of free or random movements" in Chapter II was applied; that is those individuals which deviated within ±24° from the given direction at the given angles 0° and 180° respectively and within ±12° at each of the given angles 45°, 90° and 135° were taken as "indifferent." For instance, at the given angle 90°, when the worms were tested at a distance of 1 meter from the light source, 8 positively phototactic individuals showed smaller occupied angles than 78° (90°-12°) and 80 negatively phototactic individuals larger occupied angles than 102° (90°+12°) while the remaining 12 indifferent individuals showed occupied angles in the limit from 78° to 102°. At the given angle 0° the number of individuals which showed indifferent phototaxis naturally coincides with that of the positive ones and at the given angle 180° with that of the negative ones.

At the given angle 90°, in the part of the distribution of worms we notice that the number of individuals at a distance of 1 meter from the light source is maximum in the section 136°-180°, and it

TABLE 14.

Given angles	Distances from the light source	Distribution of worms				Phototaxis		
		0°-45°	46°-90°	91°-135°	136°-180°	Positive	Indifferent	Negative
0°	1 m.	68	12	6	14	52		48
	2 m.	70	20	5	5	48		52
	3 m.	62	8	4	6	60		40
	4 m.	74	18	4	4	56		44
	Dark place	80	18	2	0	54		46

45°	1 m.	32	22	10	36	22	18	60
	2 m.	30	36	15	19	19	21	60
	3 m.	48	20	12	20	24	32	44
	4 m.	44	32	14	10	26	36	38
	Dark place	59	36	4	1	38	33	29
90°	1 m.	4	8	34	54	8	12	80
	2 m.	2	22	32	44	15	19	66
	3 m.	10	22	26	42	28	10	62
	4 m.	14	22	38	26	34	8	58
	Dark place	11	40	40	9	34	34	32
135°	1 m.	0	4	16	80	6	16	78
	2 m.	0	3	21	76	16	16	68
	3 m.	0	6	10	84	14	14	72
	4 m.	2	6	22	70	22	28	50
	Dark place	0	6	46	48	32	36	32
180°	1 m.	0	0	8	92	22	78	
	2 m.	0	0	6	94	26	74	
	3 m.	0	0	8	92	26	74	
	4 m.	0	4	8	88	28	72	
	Dark place	0	1	10	89	39	61	

decreases with the decreased intensity of light while in other sections the number of individuals tends to increase with the decreased intensity of light till in a dark place the sum of individuals in the sections 0°–45° and 46°–90° is nearly equal to the sum of individuals in the sections 91°–135° and 136°–180°; and in the part of the phototaxis it is seen that the number of the negatively phototactic individuals decreases while that of the positively phototactic ones increases in accordance with the increase of the distance from the light source, till in the dark place each number balances. Nearly the same change is seen also at the given angles 45° and 135°. These facts show that, at the given angles 45°, 90° and 135°, with the decreasing intensity of light the number of the negatively phototactic individuals decreases and in the dark place it becomes equal to the number of the positively phototactic individuals, and that when the worms were put under the influence of the stronger light many of them are forced to show a strong negative phototaxis while under the influence of the weaker light the worms cease to show a strong phototaxis.

At the given angle 0°, in the part of the distribution of worms it is seen that the number of individuals which crawled into the sections 91°–135° and 136°–180° tends to decrease with the decreased intensity of light, while the number of individuals found in the section 0°-45° tends to increase with the decreased intensity of light, and the numbers of individuals in these sections approximate to those found in the test of the random movements which was carried on in the dark place; and in the part of the phototaxis it is seen that the number of individuals which showed the negative phototaxis is nearly equal to the sum of the indifferent and positively phototactic individuals. These facts show that at the given angle 0° nearly the half number of individuals crawled indifferently near the given direction in spite of the different intensity of light, though the worms which showed the strong negative phototaxis decreased following the decreased intensity of light. This movement of the worms just stated seems to me very remarkable and seems to suggest that when the worms are put toward the light, some of them may show the positive phototaxis. The same phenomena may occur even at the given angle 45°, especially under the influence of a strong light, for the number of the positively phototactic individuals is greater in comparison with that at the given angles 90° and 135°.

At the given angle 180°, in the part of the distribution of worms it is seen that when the worms were put at the distances of 1, 2 and 3 meters respectively none was in the section 46°–90°, though at 4 meters and in the dark place a few worms were found there; and in the part of the phototaxis it is seen that the decreased intensity of light gives a smaller number of the negatively phototactic individuals and in turn increases the number of the positively phototactic individuals, and the numbers approximate to those found in the test of the random movements which were carried on in the dark place. These facts show that when the worms were exposed to the strong light many of them were forced to crawl nearer the given direction, while under the influence of the weaker light they moved freely even toward the light.

Another experiment was carried on in the same dark place which was used in connection with the experiment in Chapter II. 50 worms were brought out from a reservoir and were put together in a glass pot, in which they coiled into a mass. After the room was made dark, a few drops of the solution of methylene blue were dropped on the mass and then the glass pot was upset to put the mass of the worms on the middle of a large paper which was previously well moistened. 20 warms crawled out toward the light source forming groups of two or three worms and 7 worms crawled away from it, while 23 worms remained in the mass. Since the closure of the light source was not perfect, the result from the experiment might be considered as the case that the worms were positively phototactic to the faint light. The movement just stated, however, must be only one case of random movements because under the same condition the worms can move equally toward and away from the light source as was already stated in Chapter II. Hess (1924) says on observing the first movements of the anterior end of the body of Lumbricus terrestris under the influence of a horizontal light of 0.00118 metercandles that he could confirm the results obtained by Adams (1903). According to his table, however, the frequency of movements toward the light was 56.15% while that of movements away from it was 43.85%. This fact seems to me rather to show that the worms made random movements under the influence of the faint light which was used by Hess.

Summarizing the above statements it seems to be true that under the influence of a comparatively low intensity of light the worms can make several random movements, however that the number of individuals which change the phototaxis to the negative increases as the intensity of the light becomes stronger.

In Table 15 the occupied angles are gathered on the left and the moved angles on the right side. In the moved angles those values with - sign denote that the worms moved away from the light and the others toward it. The value of the angles which were obtained from the experiment in the dark place are taken as the average limit of the random movements discussed in Chapter II.

At a glance at the table it is seen in each set of the occupied angles that a greater distance from the light source tends to decrease the magnitude of the occupied angle till the value approaches that of the random movements. This fact shows that the increased intensity of light tends to exaggerate the negative phototaxis of the worms, and in terms of moved angles the fact is expressed thus: the decreased

TABLE 15.

	Given angles	Occupied angles					Moved angles				
		1 m.	2 m.	3 m.	4 m.	Dark place	1 m.	2 m.	3 m.	4 m.	Dark place
		in deg.	in deg.	in deg.	in deg.	in deg.	in deg.	in deg.	in deg.	in deg.	in deg.
Average angles	0°	45.34	39.83	34.42	33.84	24.00	-45.34	-39.83	-34.42	-33.84	-24.00
	45°	94.40	80.08	70.68	62.86	45.00	-49.40	-35.08	-25.68	-17.86	0.00
	90°	137.04	127.46	115.48	101.00	90.00	-47.04	-37.46	-25.48	-11.00	0.00
	135°	161.92	155.18	151.70	139.42	135.00	-26.92	-20.18	-16.70	-4.42	0.00
	180°	164.76	163.80	160.74	159.86	156.00	15.24	16.20	19.26	20.14	24.00
Positive angles	0°	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	45°	38.16	38.85	36.26	36.04	33.00	6.84	6.15	8.74	8.96	12.00
	90°	85.52	85.55	80.06	74.64	78.00	4.48	4.45	9.94	15.36	12.00
	135°	131.52	129.25	128.46	123.42	123.00	3.48	5.75	6.54	11.58	12.00
	180°	164.76	163.80	160.74	159.86	156.00	15.24	16.20	19.26	20.14	24.00
Negative angles	0°	45.34	39.83	34.42	33.84	24.00	-45.34	-39.83	-34.42	-33.84	-24.00
	45°	101.24	86.23	79.42	71.82	57.00	-56.24	-41.23	-34.42	-26.82	-12.00
	90°	141.52	131.91	125.42	116.36	102.00	-51.52	-41.91	-35.42	-26.36	-12.00
	135°	165.40	160.93	158.24	151.00	147.00	-30.40	-25.93	-23.24	-16.00	-12.00
	180°	180.00	180.00	180.00	180.00	180.00	0.00	0.00	0.00	0.00	0.00

intensity of light decreases the magnitude of the negatively moved angles and in turn increases that of the positively moved angles till these values approach those given in the random movements. It follows then that when the worms are influenced by the horizontal light they tend to occupy larger negatively and smaller positively moved angles than in the case of the random movements.

Hereafter the positively or the negatively moved angle in the dark place (±12°, see Chapter II) is called the *positive* or the *negative random angle* and is denoted by r to distinguish it from the *positively* or the *negatively moved angle* in the light which is denoted by pm or nm (in absolute values) respectively. The difference between the positive random and the positively moved angles ($r-pm$) is called the *positive forced angle* and is denoted by pf, and the difference between the negatively moved and the negative random angles ($nm-r$) is called the *negative forced angle* and is denoted by nf. So the relations between those angles may be expressed by the equations:

$$r-pm=pf$$

and $$nm-r=nf.$$

Let us suppose now that in the dark place the degrees of positive and negative phototaxis of the worms are balanced, but in the light the balance is no longer kept and the degree of positive phototaxis becomes weakend while that of negative phototaxis becomes strengthened. The weakened degree of positive phototaxis is expressed by the positive forced angle pf, and the strengthened degree of negative phototaxis by the negative forced angle nf. For an actual example, at the given angle 45° and at the distance of 1 meter the average moved angle is 49.40°, the positively moved angle 6.84° and the negatively moved angle 56.24°. Then

$$r-pm=12.00°-6.84°=5.16°=pf \dots\dots\dots\dots\dots\dots \text{I}$$

$$nm-r=56.24°-12.00°=44.24°=nf \dots\dots\dots\dots\dots\dots \text{II}$$

Add I and II. Then we obtain

$$nm-pm=56.24°-6.84°=44.24°+5.16°=nf+pf$$

or $$nm-pm=49.40°=49.40°=nf+pf.$$

According to the definition $nm-pm$ is the average moved angle or 49.40° and it is equal to $nf+pf$. Hereafter $nf+pf$ is called the *average forced angle* and is denoted by af. So in this case the average moved angle is directly the average forced angle. The same relations between the angles are found also at the given angles 90° and 135°. Since at the given angle 0° the positive random and the positively moved angles are 0°, only the equation $nm-r=nm-24°=nf$ can exist. For the same reasons at the given angle 180° the equation $r-pm=24°-pm=pf$ alone remains.

The average, positive and negative forced angles calculated from Table 15 are gathered together in Table 16. In this table, since at the given angle 90° and at the distance of 4 meters the magnitude of the positively moved angle surpasses that of the positive random angle, the positive forced angle at that point is conveniently made 0°. It is seen in the table at a glance that the magnitude of either the average,

TABLE 16.

	Given angles	1 m.	2 m.	3 m.	4 m.	Dark place
		in degrees	in degrees	in degrees	in degrees	in degrees
Average forced angles	0°	21.34	15.83	10.42	9.84	0.00
	45°	49.40	35.08	25.68	17.86	0.00
	90°	47.04	37.46	25.48	14.36	0.00
	135°	26.92	20.18	16.70	4.42	0.00
	180°	8.76	7.80	4.74	3.86	0.00
Positive forced angles	0°	0.00	0.00	0.00	0.00	0.00
	45°	5.16	5.85	3.26	3.04	0.00
	90°	7.52	7.55	2.06	0·00	0.00
	135°	8.52	6.25	5.46	0.42	0.00
	180°	8.76	7.80	4.74	3.86	0.00
Negative forced angles	0°	21.34	15.83	10.42	9.84	0.00
	45°	44.24	29.23	22.42	14.82	0.00
	90°	39.52	29.91	23.42	14.36	0.00
	135°	18.40	13.93	11.24	4.00	0.00
	180°	0.00	0.00	0.00	0.00	0.00

positive or negative forced angle decreases in accordance with the increase of the distance from the light source till it becomes 0°.

Since some discrepancies are found among the positive forced angles, it will be advantageous for us to study at first in the following pages the changes in the average and negative forced angles and then we will attempt to interprete the meaning concerning the state of the changes in the positive forced angles.

To study differential reaction of the worms against the horizontal light of constant intensity but at different given angles, Table 17 was made from Table 16. For the convenience of investigation, each average or negative forced angle at the given angle 0° was preferred as the standard of comparison, and the ratios between it and the corresponding angles at other given angles were calculated as are shown in the table. It is seen in the table that the worms react against a light of the same intensity most feebly at the given angle 180° and most strongly not at the given angle 0° as might be anticipated but at 45° and 90°, and that at the same given angle the ratios are nearly equal in spite of the different distances from the light source, except at the distance of 4 meters where the ratios are com-

TABLE 17.

	Given angles	1 m	2 m.	3 m.	4 m.	averages
Ratios between the average forced angles, those at 0° being made as the unit.	0°	1.00	1.00	1.00	1.00	1.00
	45°	2.32	2.22	2.48	1.82	2.34
	90°	2.20	2.37	2.45	1.46	2.34
	135°	1.26	1.27	1.61	0.45	1.38
	180°	0.41	0.49	0.46	0.39	0.45
Ratios between the negative forced angles, those at 0° being made as the unit.	0°	1.00	1.00	1.00	1.00	1.00
	45°	2.08	1.84	2.15	1.50	2.02
	90°	1.86	1.91	2.23	1.46	2.00
	135°	0.86	0.88	1.08	0.41	0.94
	180°	0.00	0.00	0.00	0.00	0.00

paratively small owing probably to the less intensity of the light, for under such a condition several random movements of the worms might occur. For this reason the values given in 4 m. are not included in the average shown in the same table. The average ratios between the average forced angles may be taken irrespectively with light intensity as the probable definite ratio of the degree of negative phototaxis at the given angles, when compared with the degree of negative phototaxis at the given angle 0°.

TABLE 18.

	Given angles	1 m.	2 m.	3 m.	4 m.	Dark place
Ratios between the average forced angles, those at 2 m. being made as the unit.	0°	1.36	1.00	0.66	0.63	0.00
	45°	1.41	1.00	0.73	0.51	0.00
	90°	1.26	1.00	0.68	0.48	0.00
	135°	1.33	1.00	0.83	0.22	0.00
	180°	1.11	1.00	0.61	0.49	0.00
Ratios between the negative forced angles, those at 2 m. being made as the unit.	0°	1.36	1.00	0.66	0.63	0.00
	45°	1.51	1.00	0.77	0.51	0.00
	90°	1.31	1.00	0.78	0.48	0.00
	135°	1.31	1.00	0.81	0.29	0.00
Average ratios		1.33	1.00	0.73	0.47	0.00

To study differential reactions of the worms against the horizontal light of various intensities at any given angle, Table 18 was made from Table 16. For the convenience of comparison, the average or the negative forced angle at a distance of 2 meters was preferred as the standard. For this purpose 100 worms were used at each given angle in the experiment and thus the results are most reliable. Each ratio between the standard and the corresponding angle at another distance was calculated and is arranged together in the table. Since the ratios of the negative forced angles at the given angle 180° are indeterminable, they are omitted in the table. It will be seen in the table that the shorter distance from the light source or the stronger intensity of light causes the stronger negative phototaxis of the worms, and that the same distance gives nearly equal ratio or reaction irrespective of the given angles. The average ratios which are shown in the same table may be taken as the probable definite ratio of the degree of negative phototaxis at the distances of 1, 2, 3 and 4 meters from the light source, when compared with the degree of negative phototaxis at the distance of 2 meters.

In combining the results from Tables 17 and 18 the conclusion is that the worms show the differential negative phototaxis in definite ratios against the horizontal light of various intensities at various given angles. As the probable defínte ratios, the combined ratios are given in Table 19. In this table the value at the given angle 0° and at the distance of 2 meters was taken as the standard; so the average ratios in Table 18 (1.33 : 1.00 : 0.73 : 0.47) are directly transferred to the table as the probable definite ratios at the given angle 0°, and the average ratios in Table 17 (1.00: 2.34: 2.34: 1.38: 0.45 in the average forced angles and 1.00: 2.02: 2.00: 0.94: 0.00 in the negative forced angles) as the probable definite ratios at the distance of 2 meters, and consequently the remaining values were obtained by multiplying the

TABLE 19.

	Given angles	1 m.	2 m.	3 m.	4 m.	Dark place
Combined ratios between the average forced angles, the one at 0° and 2 m. being made the unit.	0°	1.33	1.00	0.73	0.47	0.00
	45°	3.11	2.34	1.71	1.10	0.00
	90°	3.11	2.34	1.71	1.10	0.00
	135°	1.84	1.38	1.01	0.65	0.00
	180°	0.60	0.45	0.33	0.21	0.00
Combined ratios between the negative forced angles, the one at 90° and 2 m. being made the unit.	0°	1.33	1.00	0.73	0.47	0.00
	45°	2.69	2.02	1.47	0.95	0.00
	90°	2.66	2.00	1.46	0.94	0.00
	135°	1.25	0.94	0.69	0.44	0.00
	180°	0.00	0.00	0.00	0.00	0.00

value at each given angle at the distance of 2 meters with the corresponding value at each distance at the given angle 0°. When the real angular value at the given angle 0° and at the distance of 2 meters is determined, the combined ratios may be taken of course as the multiplying factors to obtain the angular values at other conditions of the light.

Applying the combined ratios the forced angles in Table 16 are revised in Table 20. In the revised table the positive forced angles are calculated in utilizing the equation $nf+pf=af$. The following facts may be seen in the table.

1. The positive forced angle increases the magnitude in accordance

TABLE 20.

	Given angles	1 m.	2 m.	3 m.	4 m.	Dark place
		in degrees	in degrees	in degrees	in degrees	in degrees
Average forced angles	0°	21.05	15.83	11.56	7.44	0.00
	45°	49.26	37.04	27.04	17.41	0.00
	90°	49.26	37.04	27.04	17.41	0.00
	135°	29.07	21.86	15.96	10.27	0.00
	180°	9.47	7.12	5.20	3.35	0.00
Positive forced angles	0°	0.00	0.00	0.00	0.00	0.00
	45°	6.72	5.06	3.69	2.38	0.00
	90°	7.15	5.38	3.93	2.53	0.00
	135°	9.28	6.98	5.10	3.28	0.00
	180°	9.47	7.12	5.20	3.35	0.00
Negative forced angles	0°	21.05	15.83	11.56	7.44	0.00
	45°	42.54	31.98	23.35	15.03	0.00
	90°	42.11	31.66	23.11	14.88	0.00
	135°	19.79	14.88	10.86	6.99	0.00
	180°	0.00	0.00	0.00	0.00	0.00

with the increase of the magnitude of the given angle. Since angles at the given angle 0° or 180° are comparable always with twice the corresponding ones at others, the fact just stated shows that the positive phototaxis of the worms is most strongly suppressed when the worms are placed at the given angle 135° and the decreased magnitude of the given angle causes the more pronounced, positive phototaxis of the worms.

2. The increased distance from the light source decreases not only either magnitude of the average, positive or negative forced angle till it becomes 0° at the infinite distance or in the dark place, but the difference between the successive forced angles. The fact itself proves that the locus of each set of the forced angles at each given angle with respect to the distances from the light source is not a straight line, but that it is a curve which resembles a hyperbola.

For the purpose of ascertaining the true tendency of changes in the forced angles, following the general tendency of curves stated in the above second fact, let us utilize the equation $xy=c$ for each hyperbola with respect to the corresponding axes, x denoting the distance from the light, y the magnitude of the forced angle and c the constant, and calculate the mathematic angular values to compare with the angular values obtained in the experiments.

In the following pages the revised values of the forced angles in Table 20 are taken as the basis of calculation.

For the actual example of calculation, let us treat the case at the given angle 0°. In this case the abscissa passes the occupied angle 24°. Let the corresponding ordinate intersect with the abscissa at the distance of x from the center of the light source or 0 meter in the empirical scale. Then the following 4 points are settled: ($1+x$ m., 21.05°), ($2+x$ m., 15.83°), ($3+x$ m., 11.56°) and ($4+x$ m., 7.44°). Combining these points the average value of x 0.56 meter is obtained. So we can take

$$(x+0.56)y=c$$

as the empirical formula for the hyperbola at the given angle 0°. Let us take (1.56m., 22.10°) as the co-ordinates of a fixed point on the hyperbola. Then $1.56\times 22.10=c=34.4760$. If we divide c with the sum of 0.56 and the successive value of x (distance), we can obtain the successive value of y (forced angle).

The empirical formula $(x+0.56)y=c$ is also applicable to the hyperbolae in other cases and when a point is fixed on each hyperbola, the corresponding c is also fixed, and the successive values of y at each given angle are obtainable in the same way as in the case of the given angle 0°. The values of c which were determined by the fixed points on the hyperbolae are shown in Table 21, and the successive values of y which were calculated are shown in Table 22.

TABLE 21.

	Given angles	C	Position of abscissa in occupied angles	Fixed points
Average forced angles	0°	84.4760	24°	(1.56 m., 22.10°)
	45°	80.6832	45°	(1.56 m., 51.72°)
	90°	80.6832	90°	(1.56 m., 51.72°)
	135°	47.6112	135°	(1.56 m., 30.52°)
	180°	15.5064	156°	(1.56 m., 9.94°)
Positive forced angles	45°	10.9980	33°	(1.56 m., 7.05°)
	90°	11.7000	78°	(1.56 m., 7.50°)
	135°	15.1944	123°	(1.56 m., 9.74°)
Negative forced angles	45°	69.6852	57°	(1.56 m., 44.68°)
	90°	68.9832	102°	(1.56 m., 44.22°)
	135°	32.4168	147°	(1.56 m., 20.78°)

It will be found in the comparison of Tables 16 and 22 that the difference between the actual and calculated angles is not so large and among 44 cases, in 29 it hardly surpasses 2.5°, though in 4 it is above 5°. So it seems to me that in general the results from the calculation coincide with the actual ones from the experiments and consequently the equation $(x+0.56)y=c$ can be applied advantageously at any given angle for expressing the changes in the forced angles according to the distances from the light source.

TABLE 22.

	Given angles	1 m.	2 m.	3 m.	4 m.
		in degrees	in degrees	in degrees	in degrees
Calculated average forced angles	0°	22.10	13.47	9.68	7.56
	45°	51.72	31.52	22.66	17.70
	90°	51.72	31.52	22.66	17.70
	135°	30.52	18.60	13.37	10.44
	180°	9.94	6.06	4.36	3.40
Calculated positive forced angles	45°	7.05	4.30	3.09	2.42
	90°	7.50	4.58	3.51	2.57
	135°	9.74	5.94	4.26	3.33
Calculated negative forced angles	45°	44.66	27.22	19.57	15.28
	90°	44.22	26.94	19.15	15.13
	135°	20.78	12.66	9.11	7.11

The intensity of light is reversely proportional to the square of the distance from the light source. Let us suppose now that the present light source shows the intensity of 400 meter-candles at the distance of 1 meter (500 watts÷1.25=400 m. c., Barrows 1912). Let I be the intensity of light and x be the distance from the present light source. Then in the experiments

$$I=\frac{400}{x^2}.$$

Hence
$$x=\sqrt{\frac{400}{I}}.$$

Substitute the value of x in the equation $(x+0.56)y=c$. Then

$$\left(\sqrt{\frac{400}{I}}+0.56\right)y=c.$$

Hence
$$y=\frac{c}{\sqrt{\frac{400}{I}}+0.56}.$$

TABLE 23.

Distances from the light source	Intensity of light	Forced angles at 0°	Forced angles at 45°			Forced angles at 90°			Forced angles at 135°			Forced angles at 180°
			Positive	Average	Negative	Positive	Average	Negative	Positive	Average	Negative	
	in m. c.	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees
Dark place	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30 m.	0.44	1.13	0.36	2.64	2.28	0.38	2.64	2.26	0.50	1.56	1.06	0.51
20 m.	1.00	1.68	0.53	3.92	3.39	0.56	3.92	3.36	0.74	2.32	1.58	0.75
15 m.	1.78	2.22	0.70	5.18	4.48	0.75	5.18	4.43	0.98	3.06	2.08	1.00
10 m.	4.00	3.27	1.04	7.64	6.60	1.11	7.64	6.53	1.43	4.50	3.07	1.47
9 m.	4.94	3.61	1.15	8.44	7.29	1.22	8.44	7.22	1.61	5.00	3.29	1.62
8 m.	6.25	4.03	1.29	9.43	8.14	1.37	9.43	8.06	1.77	5.56	3.79	1.81
7 m.	8.16	4.56	1.46	10.68	9.22	1.56	10.68	9.12	2.01	6.30	4.29	2.05
—	10.00	5.00	1.59	11.69	10.10	1.69	11.69	10.00	2.20	6.90	4.70	2.25
6 m.	11.11	5.26	1.68	12.30	10.62	1.78	12.30	10.52	2.32	7.26	4.94	2.36
5 m.	16.00	6.20	1.97	14.51	12.54	2.10	14.51	12.41	2.77	8.60	5.83	2.79
—	20.00	6.86	2.19	16.04	13.85	2.32	16.04	13.72	3.03	9.47	6.44	3.08
4 m.	25.00	7.56	2.42	17.70	15.28	2.57	17.70	15.13	3.33	10.44	7.11	3.40
—	30.00	8.21	2.62	19.21	16.59	2.79	19.21	16.42	3.62	11.34	7.72	3.69
3 m.	44.44	9.68	3.09	22.66	19.57	3.51	22.66	19.15	4.26	13.37	9.11	4.36
—	75.00	11.85	3.78	27.72	23.94	4.01	27.72	23.71	4.88	16.36	11.48	5.31
2 m.	100.00	13.47	4.30	31.52	27.22	4.58	31.52	26.94	5.94	18.60	12.66	6.06
—	150.00	15.74	5.02	36.84	31.82	5.24	36.84	31.50	6.94	21.74	14.80	7.08
—	200.00	17.50	5.59	40.96	35.37	5.94	40.96	35.02	7.71	24.17	16.46	7.87
—	250.00	18.94	6.04	44.33	38.29	6.43	44.33	37.90	8.35	26.16	17.81	8.52
—	300.00	20.16	6.43	47.18	40.75	6.84	47.18	40.34	8.89	27.84	18.95	9.08
—	350.00	21.22	6.78	49.80	43.02	7.22	49.80	42.58	9.38	29.39	20.01	9.57
1 m.	400.00	22.10	7.05	51.72	44.67	7.50	51.72	44.22	9.74	30.52	20.78	9.94

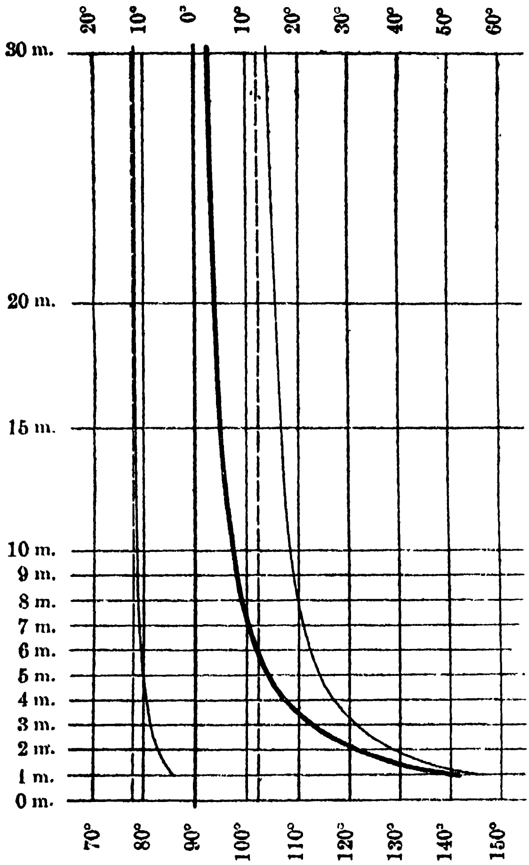


Fig. 7.

c at each given angle is shown respectively in Table 21, so if we write the value of intensity of light for *I* we obtain from the equation the corresponding value of *y* which is the magnitude of the forced angle. The forced angles calculated are arranged together in Table 23. In this table the calculation of angles is limited to the distance of 1 meter from the present light source or to the intensity of 400 meter-candles.

To show the general tendency of changes in reactions of the worms, for example, at the given angle 90° Figs. 7 and 8 were made from Table 23. In Fig. 7 the distance from the present light source and in Fig. 8 the intensity of light are scaled on abscissa; and in both figures the magnitude of angles are scaled on ordinate: the occupied angle on the left and the moved angle on the right side. The tracing of the average angles is denoted by the thick line and those of the positive and negative angles by the thin lines.

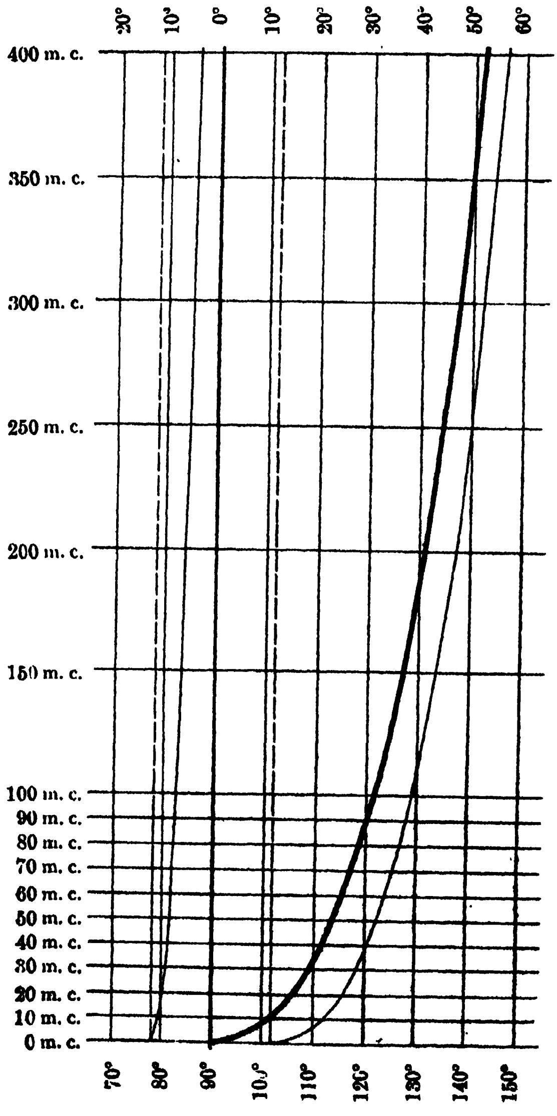


FIG. 8.

CONCLUSIONS.

1. Under the influence of the comparatively low intensity of horizontal light the worms can make several random movements, but the increased intensity tends to restrict the movements of the worms to the negative phototaxis. This conclusion coincides with Harper's on Perichaeta and Lumbricus.

2. The worms react against the horizontal light of a constant intensity in a definite ratio according to the difference of given angles (Table 17).

3. The worms react against the horizontal light at each given angle in a definite ratio according to the difference of light intensity (Table 18).

4. At each given angle the locus of the forced angles at several distances from the horizontal light source may be expressed by the hyperbolic equation

$$xy=c,$$

when x denotes the distance, y the magnitude of the forced angle and c the constant. Then the equation

$$y=\frac{c}{x}$$

shows that the amount of the reaction which is expressed by the magnitude of the forced angle is reversely proportional to the distance from the light.

5. When I denotes an intensity of light, x a distance from the light source and k the intensity at the distance x,

$$I=\frac{k}{x^2} \quad \text{or} \quad x=\sqrt{\frac{k}{I}}.$$

Substitute the value of x to the equation $y=\frac{c}{x}$ and obtain

$$y=K\sqrt{I}.$$

This equation shows that the amount of reaction against the horizontal light is proportional to the square root of the intensity of light.

6. Under some conditions the worms may be attracted by the light (Table 14, also see Chapter I). The positively moved angle at the given angle 90° and at the distance of 4 meters shows a larger

deviation than that of the positive random angle (Table 15). This behavior of the worms may be due to the fatigue previous to the experiment as for instance in a reservoir as in the case stated in Chapter I, but it is our intention to discuss this question in the Chapters VI—IX.

Chapter IV. The influence of elevated light upon the worms.

The object of the experiments was to observe the phototactic behavior when the worms were placed under the influence of an elevated light.

For the purpose of investigation an electric lamp of 500 watts was used as the light source, and 7 experiments were carried on at a distance of 2 meters from the light source at various degrees of elevation angles. In each experiment a number of the worms were individually brought out newly from a reservoir and were placed initially at the given angles 0°, 45°, 90°, 135° and 180°.

Experiment I was carried on at the elevation angle 0°. As the elevation angle 0° of the light source means, of course, a horizontal light, the data obtained from Experiment II in Chapter III were utilized for the purpose of comparison.

Experiment II was carried on at the elevation angle 15°. 50 worms were tested at each given angle and the tests were made during the latter part of September.

Experiment III was carried on at the elevation angle 25°. 50 worms were tested at each given angle and the tests were made during the latter part of September.

Experiment IV was carried on at the elevation angle 35°. 50 worms were tested at each given angle and the tests were made during the middle part of October.

Experiment V was carried on at the elevation angle 45°. 50 worms were tested at each given angle and the tests were made during the earlier part of October.

Experiment VI was carried on at the elevation angle 55°. 50 worms were tested at each given angle and the tests were made during the earlier part of October.

Experiment VII was carried on at the elevation angle 90°. The tests were made during the middle part of November. The result has already been stated in Chapter II in comparison with the case of random movements in a dark place.

The results from the above experiments were gathered together in Tables 24 and 25. In the present chapter the results from the fourth angle alone are stated.

In Table 24 the number of individuals is given in percentage value at each given angle and at each elevation angle of the light source. The table consists of two parts, one the distribution of worms and the other of the worms which exhibited the similar phototaxis (See Chapter III). In the part of the distribution of worms the angle from 0° to 180° is divided into 4 sections, viz. 0°-45°, 46°-90°, 91°-135° and 136°-180°.

At the given angle 45° in the part of the distribution of worms it is seen that in the section 136°-180° the maximum value is given at the elevation angle 25°, and in the section 0'-45° the minimum value is given at the same elevation angle. We also notice that the decreased elevation angle below 25° tends to decrease the value in the section 136°-180°, and to increase in the section 0°-45°. Similarly the increased elevation angle above 25° tends to decrease the value in the section 136°-180° and to increase in the section 0°-45°, and finally the values approach those given by the test made in random movements at the elevation angle 90° or under the influence of the overhanging light. In the part of the phototaxis it is seen that when the worms were tested at the elevation angle 25° the number of the negatively phototactic individuals is greater than at other elevation angles and the decreased or increased degree of elevation angle from 25° tends to decrease the number of individuals; the contrary statement applies to the case of the positively phototactic individuals.

At the given angle 90° we find similar relations as are given in the above.

At the given angle 0° the distribution of the worms shows more or less irregularities, but taking into consideration the results given in the phototaxis it may be stated that the worms are strongly influenced by the light at the elevation angle 25° and with either the decrease or the increase of the elevation angle from 25° the worms become

TABLE 24.

Given angles	Angles of elevation	Distribution of worms				Phototaxis		
		0°-45°	46°-90°	91°-135°	136°-180°	Positive	Indifferent	Negative
0°	5°	70	20	5	5	48		52
	15°	66	12	8	14	48		52
	25°	60	14	4	22	36		64
	35°	60	24	12	4	44		56
	45°	76	16	0	8	54		46
	55°	78	12	2	8	58		42
	90°	90	8	2	0	64		36
45°	0°	30	36	15	19	19	21	60
	15°	24	26	22	28	12	22	66
	25°	16	32	14	38	6	16	78
	35°	24	34	18	24	16	14	70
	45°	40	28	28	4	26	26	48
	55°	48	44	6	2	28	28	44
	90°	50	45	4	1	31	36	33
90°	0°	2	22	32	44	15	19	66
	15°	4	12	34	50	12	10	78
	25°	0	12	26	60	8	8	84
	35°	8	30	32	30	28	14	58
	45°	20	26	34	20	36	16	48
	55°	10	34	44	12	28	26	46
	90°	5	45	45	5	33	34	33
135°	0°	0	3	21	76	16	16	68
	15°	0	8	10	82	12	12	76
	25°	0	2	6	92	6	20	74
	35°	2	6	22	70	12	28	60
	45°	0	4	24	72	18	26	56
	55°	4	4	28	64	26	22	52
	90°	1	4	45	50	33	36	31
180°	0°	0	0	6	94	26	74	
	15°	0	8	10	82	30	70	
	25°	0	4	2	94	30	70	
	35°	0	2	10	88	30	70	
	45°	0	0	16	84	40	60	
	55°	0	0	18	82	40	60	
	90°	0	2	8	90	36	64	

less influenced by the light.

At the given angle 135° the distribution of the worms shows nearly the same tendency of changes as that at the given angles 45° and 90°. In the part of the phototaxis, however, the maximum number of the negatively phototactic individuals is given at the elevation angle 15°.

At the given angle 180° the distribution of the worms is irregular, but it tends to show that the maximum number of the negatively phototactic individuals is found at the elevation angle 0°.

In Table 25 the changes in the magnitude of angles are shown according to the elevation angles. Among the moved angles - sign denotes the moved angles away from and the others without any sign, those toward the light. The values at the elevation angle 0° at a distance of 2 meters from the light source are transferred directly from Table 23 in Chapter III and the values at the elevation angle 90° were calculated by utilizing the average limit of random movements discussed in Chapter II. The following facts may be seen in the table:

1. The average occupied angles at the given angles 0°, 45°, 90° and 135° show that the magnitude increases gradually from the elevation angle 0° to 25° and then decreases gradually in accordance with the increase of the magnitude of the elevation angle till the values approach those in the random movements.

2. At the given angle 180° the magnitude of the average occupied angles tends to decrease gradually from the elevation angle 0° to 90°.

3. The magnitude of the positive occupied angles is invariably maximum at the elevation angle 25° at the given angles 45°, 90° and 135°, though slight irregularities are shown.

4. In the negative occupied angles at the given angles 45° and 90° the maximum magnitude is found at the elevation angle 25°, but at the given angle 135°, the maximum is shown at the elevation angle 15°.

From Tables 24 and 25 it seems to me that the worms at the given angles from 0° to about 135° react most strongly against the light at the elevation angle of about 25°, but beyond 135° the worms become to react most strongly against the light at the elevation angle of a smaller degree than 25°, till at the given angle 180° the reaction becomes most pronounced at the elevation angle 0°.

TABLE 25.

	Given angles / Angles of elevation	Average angles					Positive angles					Negative angles				
		0°	45°	90°	135°	180°	0°	45°	90°	135	180°	0°	45°	90°	135°	180°
		in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.
Occupied angles	0°	37.47	76.52	121.52	135.60	162.06	0.00	37.30	82.58	128.94	162.06	37.47	84.22	128.94	159.66	180.00
	15°	52.72	94.78	128.32	155.68	160.30	0.00	41.90	84.92	128.04	160.30	52.72	97.88	133.40	162.64	180.00
	25°	60.74	106.18	139.20	158.26	160.02	0.00	42.36	87.48	132.26	160.02	60.74	108.82	141.72	161.00	180.00
	35°	45.52	90.14	108.04	147.48	156.14	0.00	·42.10	79.94	126.98	156.14	45.52	93.04	118.10	155.50	180.00
	45°	35.60	65.16	94.14	144.62	156.10	0.00	37.48	73.52	128.66	156.10	35.60	72.68	110.62	150.96	180.00
	55°	34.54	53.24	94.52	139.14	154.74	0.00	36.26	79.56	124.08	154.74	34.54	61.88	104.96	150.06	180.00
	90°	24.00	45.00	90.00	135.00	156.00	0.00	33.00	78.00	123.00	156.00	24.00	57.00	102.00	147.00	180.00
Moved angles	0°	-37.47	-31.52	-31.52	-18.60	17.94	0.00	7.70	7.42	6.06	17.94	-37.47	-39.22	-38.94	-24.66	0.00
	15°	-52.72	-49.78	-38.32	-20.68	19.70	0.00	3.10	5.08	6.96	19.70	-52.72	-52.88	-43.40	-27.64	0.00
	25°	-60.74	-61.18	-49.20	-23.26	19.98	0.00	2.64	2.52	2.74	19.98	-60.74	-63.82	-51.72	-26.00	0.00
	35°	-45.52	-45.14	-18.04	-12.48	23.86	0.00	2.90	10.06	8.02	23.86	-45.52	-48.01	-28.10	-20.50	0.00
	45°	-35.60	-20.16	-4.14	-9.62	23.90	0.00	7.52	16.48	6.34	23.90	-35.60	-27.68	-20.62	-15.96	0.00
	55°	-34.54	-8.24	-4.52	-4.14	25.26	0.00	8.64	10.44	10.92	25.26	-34.54	-16.88	-14.96	-15.06	0.00
	90°	-24.00	0.00	0.00	0.00	24.00	0.00	12.00	12.00	12.00	24.00	-24.00	-12.00	-12.00	-12.00	0.00

If we allow ourselves to think that the reactions of the worms are caused by the definite degree of the directive sensation which arose in the worms against the definite intensity of light, the statement in Chapter III, that the worms react in a definite ratio against the horizontal light of a definite intensity irrespective of the different given angles, may be interpreted as follows: the worms sensate the same intensity of the horizontal light in the same degree at each given angle. It is ascertained, however, in the present chapter that under the influence of elevated light the worms exhibit different reactions according to the difference of the given angles as well as of the elevation angles of the light source. Then what are the causes of the differential sensations which arose in the worms? The difference of the elevation angles alone must be considered as the influencing factor. Let us study the tendency of changes in the degree of directive sensation in the worms under the influence of elevated light.

The present experiments were carried on on the white papers and according to Barrows (1912) the coefficient of reflection for a white cartridge paper is 0.80, so the reflected light from the paper may influence the magnitude of the forced angles, and consequently the degree of sensations which was produced by the elevated light must be determined by the sum of the intensity of direct light and of that of reflected light. The intensity of the direct light is constant and that of the reflected light is variable at each elevation angle. Let the reflection coefficient for the experimenting paper be 0.80 and the intensity of the direct light be equal to 100 meter-candles. Then the intensity of the reflected light on the surface perpendicular to the paper and to the vertical plane which is parallel to the direction of the light rays is $(0.80 \times 100)\cos\theta$ where the elevation angle is θ.

From the relation

$$\text{direct light} + \text{reflected light} = 100 + (0.80 \times 100)\cos\theta$$

we obtain the following values for various elevation angles.

At the elevation angle 0°, since we have actually no reflected light in spite of $\cos\theta = 1$, 100 m.c. is the intensity of the influencing light.

At the elevation angle	15°	$100 + 80 \times 0.9659 = 177.27$ m.c.
	25°	$100 + 80 \times 0.9063 = 172.50$ m.c.
	35°	$100 + 80 \times 0.8192 = 165.54$ m.c.
	45°	$100 + 80 \times 0.7071 = 156.57$ m.c.

55° $100+80\times0.5736=145.89$ m.c.
65° $100+80\times0.4226=133.81$ m.c.
75° $100+80\times0.2588=120.70$ m.c.
90° $100+80\times0.0000=100.00$ m.c.

In Fig. 9 a worm at the point A is influenced by the light *l* at the elevation angle *θ* and it is assumed that the worms moved away from the light, that is to the point B. Let AB denote the magnitude

FIG. 9.

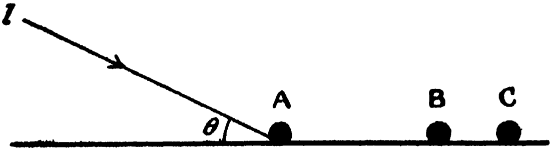


FIG. 10.

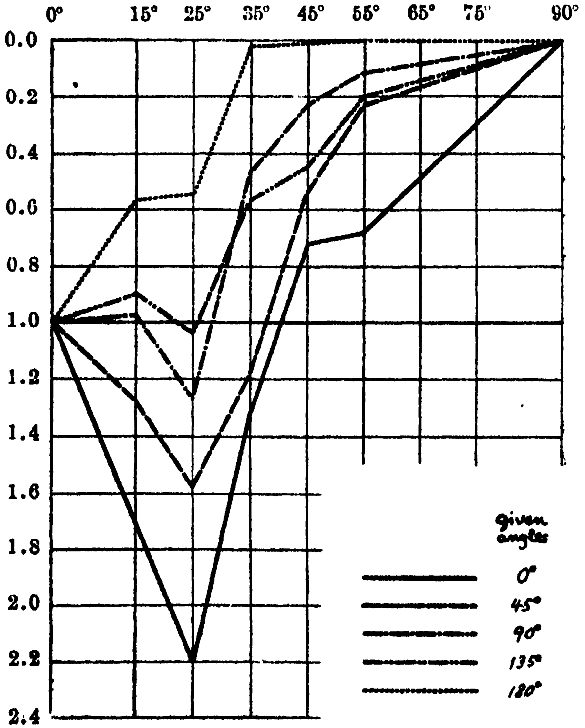


TABLE 26.

Elevation angles	Intensity of light	Given angle 0°			Given angle 45°			Given angle 90°			Given angle 135°			Given angle 180°		
		AB	AC	AB/AC	AB	AC	AB/AC	AB	AC	AB/AC	AB	AC	AB/AC	AB	AC	AB/AC
	in m. c.	in degr.	in degr.		in degr.	in degr.		in degr.	in degr.		in degr	in degr.		in degr.	in degr.	
0°	100.00	13.47	13.47	1.00	31.52	31.52	1.00	31.52	31.52	1.00	18.60	18.60	1.00	6.06	6.06	1.00
15°	177.27	28.72	16.71	1.71	49.78	39.10	1.28	38.32	39.10	0.98	20.68	23.08	0.90	4.30	7.52	0.57
25°	172.50	36.74	16.55	2.22	61.18	38.73	1.58	49.20	38.73	1.27	23.26	22.38	1.04	4.02	7.44	0.54
35°	165.54	21.52	16.30	1.31	45.14	38.09	1.19	18.04	38.09	0.47	12.48	22.03	0.57	0.14	7.33	0.02
45°	156.57	11.60	16.01	0.72	20.16	37.46	0.54	8.62	37.46	0.23	9.62	21.64	0.45	0.10	7.20	0.01
55°	145.89	10.54	15.54	0.68	8.24	36.38	0.23	4.52	36.38	0.12	4.14	21.02	0.20	0.00	6.99	0.00
90°	100.00	0.00	13.47	0.00	0.00	31.52	0.00	0.00	31.52	0.00	0.00	18.60	0.00	0.00	6.06	0.00

of the forced angle or the degree of directive sensation against the light at the elevation angle θ. When the light comes from the horizontal direction let us assume that the worm moved from the point A to the point C. Let AC denote the magnitude of the forced angle or the degree of directive sensation against the horizontal light of the same intensity as the above. Then AB/AC is the ratio between the forced angles or sensations against the elevated and horizontal lights of the same intensity or the coefficient for the degree of sensation when the light is raised to the elevation angle θ from the horizontal position.

Since as is seen in Table 25 the changes in the positive and negative angles may better be interpreted from the changes in the average angles, we shall rather study AB/AC concerning the average forced angles. In Table 25, since the positively moved angle at the given angle 90° surpasses the positive random angle 12° at the elevation angle 45°, and at the given angle 180° it surpasses the positive random angle 24° at the elevation angle 55°, these positively moved angles were taken equal to the corresponding random angle, and consequently the values of the corresponding average moved angles were also altered for the purpose of calculation of the forced angles.

The values of AB which were calculated from Table 25, the values of AC which were calculated according to the general formula

$$y=\frac{c}{\sqrt{\frac{400}{I}}+0.56}$$

which was given in Chapter III, and the values of AB/AC are given in Table 26.

To see easily the changes in AB/AC according to the difference of the elevation angles at each given angle, Fig. 10 was prepared from Table 26. In the figure the elevation angles are scaled on abscissa and the ratios on ordinate. The following facts may be seen in the figure:

1. At the given angles 0° and 45° the degree of directive sensation against the light increases in accordance with the increase of the magnitude of the elevation angle from 0° to about 25°, then decreases to 90° till it becomes 0. Similar relations are found at all other given angles. This fact seems to show that the worms sensate strongly a strong light, not because the strong light makes a strong difference of intensity at both sides of the body, but because the photoreceptors of the worms respond directly to the strong light and the specially distributed photoreceptors respond specially to the light of special conditions.

2. In general the curves reveal that the ratios become gradually smaller in accordance with the increase of the magnitude of the given angle. It is highest at the given angle 0° and is lowest at 180°.

3. At the given angle 135° the ratios at the elevation angle from about 45° to 90° are close to those at the given angle 45°. This peculiarity just stated seems due to the fact that when the light was much elevated the distinction between the given angles 45° and 135° becomes obscure in the worms and consequently the ratios become closer.

CONCLUSIONS

1. At the different directions the worms exhibit differential reactions against the light at different elevation angles.

i. When the worms are placed at the given angles from 0° to about 135°, they react most strongly against the light at the elevation angle of about 25°.

ii. When the worms are placed at the given angles which are larger than 135°, they react most strongly at the elevation angles which are smaller than 25°.

iii. When the worms are placed at the given angle 180°, they react most strongly against the light at the elevation angle 0° or against the horizontal light.

2. In general the greater the magnitude of the given angle the smaller is the degree of directive sensation of the worms.

3. At the given angle 135° the worms sensate the light at the elevation angles from about 45° to 90° nearly the same as at the given angle 45°.

Chapter V. The influence of flashlight upon the worms.

The object of the experiments was to observe how the worms would respond against stimulation by flashlight.

For the purpose of investigation a 500 watts electric lamp was put as the light source in an iron box, on one side of which was a hole. An iron plate was made to slide up and down over the hole, so that when it was lifted up the light rays would pass through the hole and illuminate each worm laterally, but when it was put down it would shut off the light quickly. The darkness in the experimenting room was such that when the eyes were trained the outlines of the white paper could dimly seen.

As the preparatory part of the experiments, each fresh worm was placed on a white paper under the influence of horizontal light at the distance of two meters from the light source and then the light was shut off to let the worm crawl freely and adapt itself to the dark. And as the proper part of the experiments, after 30 seconds the worm was exposed to the flashlight for the given number of seconds and then the light was again shut off quickly to let the worm crawl freely. To count seconds in time the sound from a metronome was utilized.

Four experiments were carried on over the months of May, June and July. In each experiment 250 worms were tested. The worms crawled so irregularly that I could not settle in the experiments any standard direction where the worms were definitely to be stimulated at the beginning of the proper part. So I have divided the field into 9 angular sections, viz. 0°-10°, 11°-33°, 34°-56°, 57°-78°, 79°-101°, 102°-123°, 124°-146°, 147°-168° and 169°-180°, and I have tried several different ways for the purpose of finding the same number of individuals in each section.

In Experiment I the worms were exposed to a one-second flashlight.

In Experiment II the worms were exposed to a five-second flashlight.

In Experiment III the worms were exposed to a ten-second flashlight.

In Experiment IV the worms were exposed to the light till the crawl had proceded outside of the definite circular area of 11 cm. radius.

For the statistical study of tracks described by the worms, the moved angles in all angular sections which were determined from the occupied directions at the end of the preparatory part were averaged. The average moved angle which was made by the sudden stimulation of the light is called the *responded angle*.

In Experiments I, II and III the average moved angle which was made by the progress of the crawl just at the removal of the light is called the *first moved angle*, the average moved angle which was made by the second progress after removal of the light is called the *second moved angle* and the average moved angle which was made by the successive progresses is called the *third moved angle*.

EXPERIMENT I. THE INFLUENCE OF A ONE-SECOND FLASHLIGHT.

In the experiment the responded angle is directly the first angle, for, according to the definition, the worms made the responded angle just when they were exposed to the flashlight, but the duration of exposure was too short to end the responded progress of the crawl and consequently the progress ended only after the removal of the light, so both the responded and first moved angles are identical in this case.

The changes in the magnitude of the angles are as follows:

	Resp.	First	Second	Third
Positively moved angles	5.58°	5.58°	6.27°	7.73
Negatively moved angles	−14.59°	−14.59°	−12.90°	−12.43°
Average moved angles	−9.01°	−9.01°	−6.63°	−4.70°

According to the results in each kind of the moved angles it is seen that after the removal of the light, the worms tend to crawl toward the light source during the progress of the crawl, though the worms respond in general negatively to the sudden stimulation of the light.

The changes in the number of individuals are as follows:

> Resp.	66.	> Second	89.	> Third	115.
< Resp.	130.	< Second	57.	< Third	101.
= Resp.	54.	= Second	104.	= Third	34.
	250.		250.		250.

(Those worms which crawled further toward the light source at the following angle are denoted by >, those which crawled further away from the light source by <, and those which crawled straight successively by =. If the number of individuals which crawled toward the light source is greater than that of individuals which crawled away from it, the conclusion may be made that the worms crawl toward the light source, and *vice versa.*)

The conclusion from the data given above coincides with that from the magnitude of the angles. Moreover the difference between the number of individuals which crawled toward the light source and that of individuals which crawled away from it becomes smaller at the third moved angle than at the second, and at the third moved angle the latter tends to become equal to the former. The relation just stated shows that the worms tend to show random movements at the third moved angle.

EXPERIMENT II. THE INFLUENCE OF A FIVE-SECOND FLASHLIGHT.

In the experiment the responded angle is separated from the first moved angle.

The changes in the magnitude of the angles are as follows:

	Resp.	First	Second	Third
Positively moved angles	4.71°	4.65°	5.24°	5.77°
Negatively moved angles	-20.75°	-15.50°	-13.94°	-14.51°
Average moved angles	16.04°	-10.85°	-8.70°	-8.74°

In the positively moved angles it is seen that the worms tend to crawl toward the light source during the progress of the crawl, though at the first angle they tend to crawl away from the light source.

In the average moved angles as well as in the negatively moved angles it is seen that the worms tend to crawl toward the light source except at the third moved angle in which they tend to crawl away from it.

The changes in the number of individuals are as follows:

>Resp.	76.	>First	99.	>Second	113.	>Third	99.
<Resp.	138.	<First	74.	<Second	82.	<Third	96.
=Resp.	36.	=First	77.	=Second	55.	=Third	55.
	250.		250.		250.		250.

Except at the responded angle the number of individuals which crawled toward the light source is greater than that of individuals which crawled away from it.

At the first moved angle the fact coincides with that from the magnitude of the average and negatively moved angles.

At the second moved angle the fact coincides with that from the magnitude of all kinds of moved angles.

At the third moved angle the fact coincides only with that from the magnitude of the positively moved angles.

So, as a whole, we may conclude that the worms respond negatively to the sudden stimulation of light, but after the removal of the light the worms tend to crawl toward the light source though at the third moved angle the direction of the crawl appears indeterminable. At the third moved angle the difference between the number of individuals which crawled toward the light source and that of individuals which crawled away from it is very small showing that the direction of crawl is actually indeterminable or that the worms make random movements.

Experiment III. The influence of a ten-second flashlight.

In this experiment the responded angle is also separated from the first moved angle, as will be seen from the changes in the magnitude of the angles which are given below.

	Resp.	First	Second	Third
Positively moved angles	3.73°	3.61°	3.98°	6.11°
Negatively moved angles	-21.52°	-18.80°	-17.25°	-17.12°
Average moved angles	-17.79°	-15.19°	-13.27°	-11.01°

In the positively moved angles it is seen that at the first moved angle the worms tend to crawl away from the light source, but later they tend to crawl toward it in accordance with the progress of the crawl.

In the average and negatively moved angles it is seen that the worms tend to crawl toward the light source during the progress of the crawl.

The changes in the number of individuals are as follows:

>Resp.	54.	>First	100.	>Second	100.	>Third	115.
<Resp.	158.	<First	86.	<Second	80.	<Third	82.
=Resp.	38.	=First	64.	=Second	70.	=Third	53.
	250.		250.		250.		250.

Except at the responded angle the number of individuals which crawled toward the light source is greater than that of individuals which crawled away from it, and the fact coincides with that from the magnitude of the angles except in the case of the first positively moved angle. So, as a whole, we may conclude that the worms respond negatively to the sudden stimulation of light, but after removal of the light the worms tend to crawl toward the light source during the progress of the crawl.

Experiment IV. The influence of the sudden and continuous stimulation by the light.

In the experiment the responded angle was made by the first progress of the definitive crawl after the sudden stimulation. The first moved angle was made by the second progress. The second moved angle was made by the third, or, when the track is straight, by the third and successive progresses. And the third moved angle was made by the track which was just passing the border line of the circular area, the radius of which was 11 cm.

The changes in the magnitude of the angles are as follows:

	Resp.	First	Second	Third
Positively moved angles	5.20°	4.30°	4.32°	5.27°
Negatively moved angles	-23.94°	-25.85°	-28.68°	-35.05°
Average moved angles	-18.74°	-21.55°	-24.36°	-29.78°

In the positively moved angles it is seen that at the first moved angle the worms tend to crawl away from the light, but later they begin to crawl toward it.

In the average moved angles as well as in the negatively moved angles it is seen that the worms tend to crawl away from the light during the progress of the crawl.

The changes in the number of individuals are as follows:

>Resp.	56.	>First	86.	>Second	80.	>Third	78.
<Resp.	164.	<First	108.	<Second	116.	<Third	126.
=Resp.	30.	=First	56.	=Second	54.	=Third	46.
	250.		250.		250.		250.

The number of individuals which crawled away from the light is greater than that of individuals which crawled toward it.

So, as a whole, we may conclude that the worms tend to crawl away from the light during the progress of the crawl.

COMPARISON OF THE RESULTS FROM THE EXPERIMENTS.

I) When the worms were stimulated suddenly by the light they responded with the sudden retraction of or with the sudden turning of the anterior portion of the body, or with the continuous forward crawling. The percentage values of individuals which showed each kind of prompt movements stated in the above are given in Table 27. In the table the occupied angles from 0° to 180° in which the worms were stimulated suddenly by the light were advantageously divided into 5 angular sections, viz. 0°–22°, 23°–67°, 68°–112°, 113°–157° and

158°-180°. It may be seen from the table that the worms respond differently against the sudden stimulation of light according to the magnitude of the occupied angles in which they were stimulated. The larger magnitude of the stimulated occupied angle decreases the frequency of the retraction of the anterior portion of the body, but tends to increase the frequency of the continuous forward crawling. The frequency of the turning of the anterior portion of the body is maximum in the section 68°-112° and tends to decrease with the increasing or the decreasing magnitude of the stimulated occupied angle.

TABLE 27.

	Angular sections for stimulated angles / Duration of exposure	0°-22°	23°-67°	68°-112°	113°-157°	158°-180°
Retraction	1 second	33.3	21.6	21.8	14.7	2.3
	5 seconds	32.5	18.2	13.8	8.5	6.5
	10 seconds	19.6	19.2	14.3	2.2	0.0
	Long continued	31.9	25.0	13.0	12.3	2.5
	Average	29.3	21.0	15.5	9.5	2.8
Turning	1 second	29.2	43.2	46.8	36.1	34.9
	2 seconds	43.5	56.1	57.8	46.8	23.9
	10 seconds	52.1	42.4	48.2	32.6	28.0
	Long continued	31.9	36.6	54.3	49.1	42.5
	Average	39.2	44.6	51.8	41.1	32.3
Progression	1 second	37.5	35.2	31.9	49.2	62.8
	5 seconds	24.0	25.7	28.9	44.7	69.6
	10 seconds	28.3	38.4	37.5	65.2	72.0
	Long continued	36.2	38.4	32.7	38.6	55.0
	Average	31.5	34.4	32.7	49.4	64.9

II) Comparison of the responded angles.

	1 sec.	5 sec.	10 sec.	Long continued
Positively moved angles	5.58°	4.71°	3.73°	5.20°
Negatively moved angles	-14.59°	-20.75°	-21.52°	-23.94°
Average moved angles	-9.01°	-16.04°	-17.97°	-18.74°

In the above data the magnitude of the average and negatively moved angles apparently increases with the increasing duration of the exposure. This is due to the fact that while the worms were making the responded angles the successive stimulation of the light caused the magnitude of the angles to increase, especially when the worms showed the turning movements after the retraction of the anterior portion of the body.

III) Comparison of the average moved angles in relation to the duration of exposure to the light.

In the one-second exposure the responded angle is identical with the first moved angle, but in the five- or ten-second exposure the two angles become separate and the difference between them may be considered as the moved angle which was made by the exposure to the light for the given duration.

	Responded angle	First moved angle	Difference
Five-second exposure	16.04°	10.85°	5.19°
Ten-second exposure	17.79°	15.19°	2.60°

In the long continued exposure the third moved angle may be considered as the moved angle which was made after exposure to the light for a certain duration, and consequently the difference between the responded and the third moved angles may be taken also as the moved angle in relation to the duration of exposure to the light.

	Responded angle	Third moved angle	Difference
Long continued exposure	18.74°	29.78°	-11.04°

In the above differences, those values without any sign denote that the moved angle was smaller than the responded angle or the worms showed restoring movements, and the other with - sign denotes that the moved angle was larger than the responded angle or that the worms crawled further away from the light.

If we suppose that on an average one progress of crawl is made in 3 seconds and measures 1 cm., to crawl 11 cm. 33 seconds must be needed. If we assume 33 seconds as the duration of exposure in the long continued case and record the above differences on ordinate and the seconds on abscissa, the three points come to lie nearly on a straight line. So let us suppose advantageously that the changes of the difference (y) in relation to the duration of exposure (x) may be expressed approximately by the equation

$$2.59x + 5.00y = 38.90$$

which joins the points (5.00 sec., 5.19°) and (10.00 sec., 2.60°). Then we may infer the following relations.

1. In the equation, when $x = 0.00$ sec., $y = 7.78°$ and when $x = 1.00$ sec., $y = 7.26°$. But these cases can not be actual, for when $x = 0.00$ sec. the worms are not stimulated by the light and when $x = 1.00$ sec. the responded angle is not separated from the first moved angle.

2. When $y = 0.00°$, $x = 15.02$ sec. The result shows that when the worms were exposed to the light for about 15 seconds they crawl in the same direction with that of the responded angle, but when they were exposed for a shorter duration than 15 seconds they tend to crawl toward the light, and when they were exposed for a longer duration than 15 seconds they tend to crawl away from the light.

3. When $y = -11.04°$, $x = 36.33$ sec. The value of x is the duration of exposure in the long continued case and is approximately identical with 33 seconds which was previously supposed.

4. The state of changes in the equation $2.59x + 5.00y = 38.90$ may be expressed in other words as follows: When the duration of exposure varies the moved angles (differences between the responded and the first moved angles) are in arithmetical progression which is when $y = 0°$, $x = 15$ sec. and which increases or decreases by the common difference 0.52° for the increase or decrease of one second. In general, let the worms crawl under the influence of the horizontal light for s seconds and make the first moved angle a. If the worms made previously the responded angle r, the difference $a - r$ increases or decreases by the common difference of $\frac{a-r}{s-15}$ for the increase or decrease of one second when $s \neq 0$ and $s \neq 15$. Let us denote $\frac{a-r}{s-15}$ by y, $a - r$ by Y, and the duration of exposure by T. Then

$$yT = Y.$$

From the relation $y = k\sqrt{I}$ which gives the intensity of light in terms of y denoting the forced angle, I the intensity of light and k the constant (Chapter III), the value of y is substituted for y in the above equation $yT = Y$ and we obtain

$$kT\sqrt{I} = Y$$

or

$$T\sqrt{I} = K.$$

So the Bunsen-Roscoe Law which is expressed by the equation $TI = K$ is not applicable to the case of Allolobophora.

5. The fact, that when the worms were exposed to the light for a shorter duration than 15 seconds they tend to crawl toward the light source, is due in my opinion to the restoring movements which have been already stated in Chapter II.

IV). Comparison of the average moved angles which were made after removal of the light.

In the one-second exposure the first moved angle must be formed under the influence of the light, and even in the case of the five- or ten-second exposure the first moved angle must be made also under the influence of the light. Thus the first moved angle must be considered as being influenced by the light, but the second moved angle was not directly influenced by the light, and consequently the difference between the first and the second moved angles may be considered as the moved angle which was made after the removal of the light.

	First moved angle	Second moved angle	Difference
One-second exposure	9.01°	6.63°	2.38°
Five-second exposure	10.85°	8.70°	2.15°
Ten-second exposure	15.19°	13.28°	1.91°

According to the differences it is seen that when the worms were exposed to the light for a longer duration the difference becomes smaller, showing that the longer duration of exposure tends to inhibit the crawling toward the light source. This fact seems to suggest that, even after the removal of the light, the worms are still under the influence of the light and show the negative phototaxis against the removed light.

If we scale the differences on ordinate and the seconds on abscissa, it is obvious that the three points lie just on a straight line. From the points (1.00 sec., 2.38°), (5.00 sec., 2.15°) and (10.00 sec., 1.91°), in combination with each other, we obtain the equation

$$0.94x + 18.00y = 43.59.$$

The calculated differences from the above equation are nearly identical with the observed differences as will be seen below:

	Difference
One-second exposure	2.37°
Five-second exposure	2.16°
Ten-second exposure	1.90°

1. In the above equation, when $y = 0.00°$, $x = 46.73$ sec. The result shows that when the light was removed after stimulating the worms for about 47 seconds, the second moved angle becomes equal to the first moved angle, and a shorter duration of exposure than 47 seconds gives a smaller and a longer duration of exposure than 47 seconds a larger second moved angle than the first moved angle.

2. When the duration of exposure varies the moved angles formed by the after-effect (differences between the first and second angles) are in arithmetical progression which is when $y = 0°$, $x = 46.73$ sec. and which increases or decreases by the common difference 0.052° for the increase or decrease of one second. The common difference 0.052° may be considered as the negatively moved angle which is the after-effect of the horizontal light of 100 meter-candles for the exposure of one second and it is identical with 1/10 of the negatively moved angle which is effected by the direct light.

V). In the case of the ten-second exposure we have seen that at the third moved angle the worms crawled still further toward the light source than at the second moved angle, showing that at the third moved angle they were not effected by the removed light to show negative phototaxis. It follows then that in the case of the one- or five-second exposure the negative phototaxis which is caused by the after-effect also can not occur at the third moved angle. Actually it was suspected in these cases that the worms might show random movements at the third moved angle.

	Second moved angle	Third moved angle	Difference
One-second exposure	6.63°	4.70°	1.93°
Five-second exposure	8.70°	8.74°	-0.04°
Ten-second exposure	13.28°	11.01°	2.27°

In the differences no regular changes are to be found owing to the after-effect of the removed light. So we may conclude that the worms are stimulated by the removed light or the after-effect of light till the beginning or the completion of the second progress of the crawl, and later they begin to show random movements. If so, the duration of the continuation of the after-effect of the removed light may be considered to be within 6 seconds, and it follows that 30 seconds, for which the worms were allowed to crawl in the dark as the preparatory part of the experiments, was enough for the worms to adapt themselves to the dark.

CONCLUSIONS.

1. When the worms are stimulated suddenly by the horizontal light of 100 meter-candles, the majority show negative phototaxis.

2. The worms respond differently against the sudden stimulation of the light according to the directions against the light source (occupied angles) at where they were stimulated. The frequency of sudden retraction decreases and that of continuous forward crawling increases with the increasing magnitude of the stimulated occupied angle.

3. The amount of reaction of the worms (negative forced angle) against the light is proportional to the duration of exposure (seconds).

4. Let the duration of exposure be T, the intensity of light be I. Then

$$T\sqrt{I} = K.$$

5. The worms show after-effect from the removed light for about 6 seconds, and later they show random movements.

Chapter VI. The influence of light upon the worms with the brain removed.

We have seen in the former experiments that some of the worms showed strongly positive phototaxis, and it is the purpose of the following experiments to throw some light on this peculiar behavior.

We have already written about the positive phototaxis of the fatigued worms in Chapter I and when the worms were brought out from fresh manures in which they live, they appeared to show a more or less postive phototaxis, especially after rain when the manure was strongly fermenting, the positive phototaxis was more pronounced. Moreover the phototaxis of the worms appeared to differ greatly according to the difference of the season, that is in summer time the positive phototaxis is more pronounced than in winter time. This peculiar behavior seemed to me to suggest that those worms whose brains do not function are rather attracted by the light. We recall here the fact that when the worms were put toward either a horizontal or an elevated light at the given angles from 0° to probably 45° or more, some worms were attracted by the light, while when they were put at the given angle 135° they crawled away from the light. If negative phototaxis was the proper nature of the worms, then they ought not behave in such manner. This behavior seemed to me causally related with the state of nervous function and in order to test this hypothesis the observation on the movements of the worms, from which the anterior 3-4 segments were removed, was undertaken not knowing at that time that Hess (1924) had worked on the same point of investigation.

For the purpose of investigation an electric lamp of 500 watts was used as the light source, and 6 experiments were carried on at several distances from the light source. In each experiment a number of worms, from which the anterior 3-4 segments were removed, were placed at the given angles 0°, 45°, 90°, 135° and 180°. Each worm was brought out newly from a reservoir. In the present chapter the results from the fourth angle alone are stated though all other angles were determined as in the case of Chapter I.

Experiment I was carried on at a distance of 2 meters from the light source at the elevation angle 25°. 50 worms were tested at each given angle and the tests were made during the middle part of October.

Experiment II was carried on at a distance of 1 meter from the horizontal light source. 50 worms were tested at each given angle and the tests were made during the earlier part of August.

Experiment III was carried on at a distance of 2 meters from the horizontal light source. 50 worms were tested at each of the given angles 0°, 45°, 135° and 180° during the middle part of July. At the given angle 90°, 30 worms were tested during the latter part of June and 70 worms during the middle part of July.

Experiment IV was carried on at a distance of 3 meters from the horizontal light source. 50 worms were tested at each given angle and the tests were made during the latter part of July.

Experiment V was carried on at a distance of 4 meters from the horizontal light source. 50 worms were tested at each given angle and the tests were made during the latter part of July.

Experiment VI was carried on in the dark room in the same way as in Chapter II. Only 25 worms were tested at the given angle 90° and the tests were made during the early part of August.

Let us study at first how the worms, from which the anterior 3-4 segments were removed, will move their bodies when exposed to the light.

Since the worms are naturally more or less contracted when placed on the paper, at the beginning of the actual crawling some of them elongate the bodies at both ends at the same time, though such cases were very rare and I found only 39 cases among 1300 worms tested. In the majority of cases the worms move either the posterior or the anterior end of the body. In fact 39% moved the

TABLE 28.

Given angles Condition of light	0°	45°	90°	135°	180°
25°	80	50	54	42	50
1 m.	60	36	40	52	30
2 m.	78	38	61	36	68
3 m.	38	30	24	8	12
4 m.	24	20	16	14	14

posterior end and 61% moved the anterior end of the body. The number of individuals which began to elongate the posterior end is shown in percentage in Table 28. From the table it may be seen that the number of individuals which moved the posterior end is maximum at the given angle 0°, and that in general the decreased intensity of light tends to decrease the percentage values.

Before the definitive crawling, some worms showed preliminary elongations of the anterior or of the posterior end of the body alternately or rarely of both ends synchronously, but the majority of the worms crawled out either backward or forward directly without preliminary movements. The number of individuals which crawled

TABLE 29.

	Given angles / Condition of light	0°	45°	90°	135°	180°
Number of worms directly crawled forward.	25°	16	44	38	56	46
	1 m.	36	60	54	46	52
	2 m.	12	34	26	32	22
	3 m.	60	66	68	84	88
	4 m.	66	72	68	76	84
Number of worms crawled forward or backward with preliminary movements.	25°	20	30	28	20	26
	1 m.	24	16	24	14	32
	2 m.	18	54	32	48	48
	3 m.	30	18	20	16	2
	4 m.	20	14	22	16	16
Number of worms directly crawled backward.	25°	64	26	34	24	28
	1 m.	40	24	22	40	16
	2 m.	70	12	42	20	30
	3 m.	10	16	12	0	10
	4 m.	14	14	10	8	0

forward or backward with or without preliminary movements is shown together in percentage in Table 29. Though there are many irregularities, the following facts may be seen in the table.

1. At the given angle 0°, when the worms were put under the influence of light at the elevation angle 25° or at the distances of 1 or 2 meters from the horizontal light source, the number of individuals which directly crawled forward is smallest, while the number of individuals which directly crawled backward is largest.

2. At the given angle 0°, when the worms were put at the distance of 3 or 4 meters from the horizontal light source, the number of individuals which directly crawled forward surpasses half the number of the worms tested, while the number of individuals which directly crawled backward surpasses hardly 14%.

3. At the given angles larger than 45°, regardless of the conditions of light, the number of individuals which directly crawled forward becomes larger, while the number of individuals which directly crawled backward becomes smaller than that at the given angle 0°.

4. When the worms were influenced by horizontal light of comparatively low intensity such as at the distances of 3 or 4 meters from the light source, the number of individuals which directly crawled forward increases while the number of individuals which directly crawled backward tends to decrease with the increasing magnitude of the given angle.

5. At a distance of 2 meters from the horizontal light source,

TABLE 30.

Given angles / Condition of light	0°	45°	90°	135°	180°
25°	64	28	38	24	32
1 m.	46	26	24	42	24
2 m.	78	40	59	42	54
3 m.	10	18	16	4	10
4 m.	16	22	18	8	2

the number of individuals which directly crawled forward is minimum at each given angle and in turn the sum of the number of individuals which directly crawled backward and that of individuals which crawled out with preliminary movements is maximum at each given angle.

The number of individuals which finally crawled backward is generally smaller than those which crawled forward and the frequency distribution is shown in percentage in Table 30. In the table the fact may be seen that when the worms were put at each given angle the number of individuals is maximum at a distance of 2 meters from the light source and amounts to nearly half the number of the worms tested. This fact shows that when the worms were put under the influence of a horizontal light of about 100 meter-candles nearly half the number crawled out either forward or backward and that a stronger or weaker light than that induces the majority of individuals to crawl forward.

Among the worms which elongate the posterior end, some elongate straight or toward the light, but the cases are comparatively rare and similarly among the worms which move the anterior end those that move straight or away from the light are also comparatively rare and both cases combined hardly surpass 8%, while the majority of individuals elongate the posterior end away or the anterior end toward the light.

Some worms crawl either forward or backward while making winding movements. It is noted in this connection that when the anterior segments of the worms were cut obliquely the projected edge at the unevenly cut end attaches to the paper and consequently the worms went round and round the attached point and produced some winding movements. Even when the segments were cut off evenly, the ventral wall at the cut end often attached itself to the paper, thus producing similar winding movements. It seems, however, that the instances mentioned above can not be all the factors which produce the winding movements, but the uneven structure or development of setae etc. must be taken into consideration and thus the true nature of the winding movements still remains unknown to me.

In most cases the worms try to attain a position parallel to the light rays in spite of crawling forward or backward with the anterior cut end toward and the posterior end away from the light: that is

Table 31.

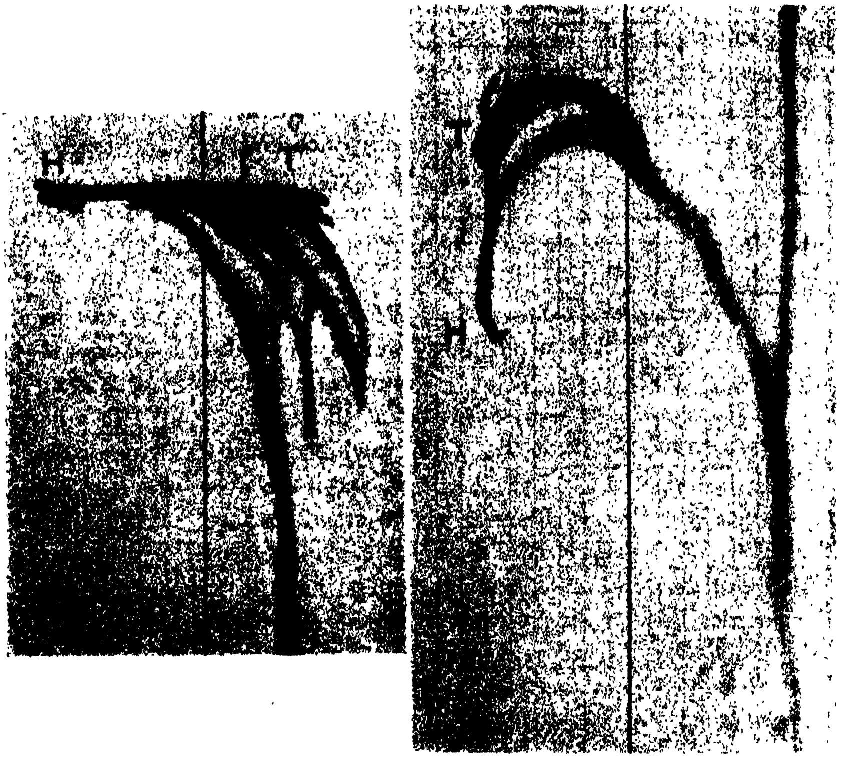
Given angles	Condition of light	Positive	Negative
0°	25°	34	64
	1 m.	48	48
	2 m.	22	78
	3 m.	84	14
	4 m.	82	18
	Dark place	100	0
45°	25°	66	30
	1 m.	68	30
	2 m.	62	38
	3 m.	80	20
	4 m.	74	26
	Dark place	86	14
90°	25°	56	40
	1 m.	68	30
	2 m.	43	56
	3 m.	74	26
	4 m.	70	30
	Dark place	50	50
135°	25°	52	46
	1 m.	58	40
	2 m.	64	32
	3 m.	76	24
	4 m.	64	36
	Dark place	14	86
180°	25°	44	50
	1 m.	42	58
	2 m.	46	44
	3 m.	60	38
	4 m.	62	38
	Dark place	0	100

to say the worms crawl to make smaller occupied angles against the light. Let us use hereafter the following technical terms: the *positive orientation* for the movements of the worms just mentioned, the *negative orientation* for the movements just in the reverse cases with the above, the *positive phototaxis* for the movements in which the worms crawl either forward or backward toward and the *negative phototaxis* for the movements away from the light.

In Table 31 the percentage values of the positively and negatively phototactic worms ars given together according to the above difinition, excluding the percentage values of the winding worms which will be seen in Table 32. For the convenience of judging the kinds of phototaxis of each worm the line passing the occupied angle 90° was taken as a border: that is the positively phototactic worm occupies the position at the lighter side near the light source and the negatively phototactic worm the position at the darker side away from the light source. The Table reveals that in general the number of the positively phototactic worms is larger than that of the negatively phototactic ones. It is worthy of note that in general when the worms crawled forward they showed positive phototaxis and when they crawled backward they showed negative phototaxis.

The preliminary positively orienting movements are much complicated when the worms make the posterior elongation. In most cases when a worm begins to elongate the posterior portion of the body away from the light, it turns the anterior cut end toward the

Fig. 11.



Tracks of two worms from which the anterior 3–4 segments were removed. 2/3 natural size. The direction of the light rays is represented by the longitudinal lines. H—anterior end, T—posterior end. In the left one the worm was placed at the given angle 90° and after making several posterior elongations it crawled backward to attain a direction parallel to the light rays with the anterior end toward the light. In the right one the worm was placed at the given angle 180° and it crawled backward just as in the left one. When the worm had shown the positively orienting nature fully its posterior end was poked with a pencil to make it crawl forward.

light its body being fixed at the given position. Elongating the posterior portion of the body away from the light as far as possible it gradually moves the body obliquely against the light. If the worm, after stretching out its body fully, is not satisfied at the occupied position, it first begins to crawl forward toward the light a little while, then stops and then begins again to elongate its posterior portion away from the light. Repeating the movements twice, thrice or many times the worm attains a position nearly parallel to the light rays and then begins the definitive backward crawling away from the light or sometimes the definitive forward crawling toward the light. In Fig. 11 actual examples of this kind of movements are shown.

It is worthy of note that among many thousands of the unoperated worms which I have tested, only a few crawled backward under the same circumstances. So the backward crawling of the operated worms must be an unusual behavior in association with the removal of the anterior 3–4 segments.

In order to understand the nature of forward and backward crawlings, I made an observation upon some worms in which the anterior 3–4 segments were removed. When a worm was crawling forward straight toward the light, if it was stimulated at the anterior end with a chop stick or a sharp pencil, it began to crawl backward with or without hesitation straight away from the light, and if the worm was stimulated at the posterior end, it began again to crawl forward straight toward the light. This process of movements can be repeated several times. The above fact appears to me to suggest that the worms from which the anterior 3–4 segments were removed only exhibit the positively orienting movements and the rapidity of attaining the parallel position with the light rays and the mode of crawling either backward or forward seem due to the difference of physiological conditions of the body against the light. If these assumptions are correct, then we do not need to distinguish the forward and backward crawling for studying the angles made by the worms against the light, for the angles made by the backward crawling are of the same nature against the light as those of the forward crawling.

The angles in the tracks described by the backward crawling worms were determined by the moving direction of the posterior ends just as in the cases of the anterior ends of the forward crawling

TABLE 32.

Given angles	Condition of light	Distribution of worms				Winding	Orientation		
		0°-45°	46°-90°	91°-135°	136°-180°		Positive	Indifferent	Negative
0°	25°	88	6	4	0	2	72		26
	1 m.	86	6	2	2	4	78		18
	2 m.	88	10	2	0	0	74		26
	3 m.	94	2	2	0	2	80		18
	4 m.	96	4	0	0	0	86		14
	Dark place	88	12	0	0	0	56		44
45°	25°	74	16	2	4	4	68	10	18
	1 m.	86	4	6	2	2	84	2	12
	2 m.	94	4	0	2	0	86	10	4
	3 m.	90	8	2	0	0	76	20	4
	4 m.	82	12	6	0	0	76	10	14
	Dark place	50	44	6	0	0	32	34	34
90°	25°	56	24	6	10	4	68	14	14
	1 m.	80	10	2	6	2	84	8	6
	2 m.	81	12	5	1	1	89	2	8
	3 m.	74	18	6	2	0	86	8	6
	4 m.	68	20	8	2	0	78	14	8
	Dark place	6	44	44	6	0	34	32	34
135°	25°	46	22	10	20	2	76	8	14
	1 m.	56	20	6	16	2	78	8	12
	2 m.	52	26	14	4	4	88	4	4
	3 m.	54	24	12	10	0	88	6	6
	4 m.	56	17	12	15	0	81	13	6
	Dark place	0	6	44	50	0	34	34	32
180°	25°	32	10	10	42	6	74	20	
	1 m	42	10	22	26	0	82	18	
	2 m.	52	16	14	8	10	88	2	
	3 m.	58	4	6	30	2	82	16	
	4 m.	52	14	10	24	0	80	20	
	Dark place	0	0	12	88	0	44	56	

worms (See Chapter I).

In a dark place even the worms from which the anterior 3–4 segments were removed always crawled forward and the average of the moved angles was ±13.58°. This value just given again supports the conclusion that ±12.00° may be taken as the average limit of random movements as in the case of the unoperated worms.

In Tables 32 and 33 are shown the orienting movements of the worms from which the anterior 3–4 segments were removed.

In Table 32 the distribution of worms, the winding and the orientation are given. The following facts may be seen in the table.

1. At each given angle, except in the case of the dark place, it is seen in the part of the distribution of worms that the number of individuals is maximum in the section 0°-45° and it is seen in the part of the orientation that the number of positively orienting individuals is far greater than that of others. From these facts I conclude that when the worms are exposed to the light, the majority of individuals exhibit the positive orientation.

2. In the part of the distribution of worms it is seen that at the given angles 45°, 90° and 135° the number of individuals which showed smaller occupied angles than the corresponding given angle is maximum at a distance of 2 meters from the light source, that at the given angle 180° the sum of individuals in the sections 0°–45°, 46°–90° and 91°–135° is maximum also at the same distance, and that either decreased or increased distance from this tends to decrease the number of individuals. In the part of the orientation it is seen that at the given angles 45°, 90°, 135° and 180° the number of positively orienting individuals is maximum at a distance of 2 meters from the light source and either decreased or increased distance decreases the number of individuals. These facts show that the positive orientation is most pronounced under the influence of the horizontal light of about 100 meter-candles (2 m. distance) and either decreased or increased intensity causes it to be less pronounced.

3. At the given angle 0°, however, it is seen in the part of the distribution of worms that the number of individuals in the section 0°-45° is maximum at a distance of 4 meters from the light source and either decreased or increased distance tends to decrease the number of individuals. We find similar phenomenon also in the part of the

TABLE 33.

	Given angles / Condition of light	Average angles					Positive angles					Negative angles				
		0°	45°	90°	135°	180°	0°	45°	90°	135°	180°	0°	45°	90°	135°	180°
		in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.
Occupied angles	25°	19.37	32.65	52.52	67.31	95.96	0.00	23.11	42.73	63.39	95.96	19.37	54.54	99.79	138.92	180.00
	1 m.	17.56	18.82	27.35	52.39	77.78	0.00	11.06	24.04	49.08	77.78	17.56	52.76	93.31	138.31	180.00
	2 m.	18.06	12.74	24.32	46.79	49.47	0.00	9.38	23.06	45.46	49.47	18.06	48.36	91.26	136.33	180.00
	3 m.	13.27	20.24	30.28	50.74	63.78	0.00	18.28	27.62	49.16	63.78	13.27	46.96	92.66	136.58	180.00
	4 m.	8.52	26.40	37.80	53.38	65.60	0.00	19.14	34.42	51.36	65.60	8.52	52.26	93.38	137.02	180.00
	Dark place	24.00	45.00	90.00	135.00	156.00	0.00	33.00	78.00	123.00	156.00	24.00	57.00	102.00	147.00	180.00
Moved angles	25°	-19.37	12.35	37.48	67.69	84.04	0.00	21.89	47.27	71.61	84.04	-19.37	-9.54	-9.79	-3.92	0.00
	1 m.	-17.56	26.18	62.65	82.61	102.22	0.00	33.94	65.96	85.92	102.22	-17.56	-7.76	-3.31	-3.31	0.00
	2 m.	-18.06	32.26	65.68	88.21	130.53	0.00	35.62	66.94	89.54	130.53	-18.06	-3.36	-1.26	-1.33	0.00
	3 m	-13.27	24.76	59.72	84.26	116.22	0.00	26.72	62.38	85.84	116.22	-13.27	-1.96	-2.66	-1.58	0.00
	4 m.	-8.52	18.60	52.20	81.62	114.40	0.00	25.86	55.58	83.64	114.40	-8.52	-7.26	-3.38	-2.02	0.00
	Dark place	-24.00	0.00	0.00	0.00	24.00	0.00	12.00	12.00	12.00	24.00	-24.00	-12.00	-12.00	-12.00	0.00

orientation. These facts show that at the given angle 0° the positive orientation is most strongly pronounced under the influence of the horizontal light of about or weaker than 25 meter-candles (4 m. distance) and either decreased or increased intensity causes it to be less pronounced.

4. The winding movements are seen only when the worms were influenced by a comparatively strong light.

In Table 33 the changes in the magnitude of the angles are shown. Among the moved angles - sign denotes the moved angles away from and others without any sign denote angles toward the light. The values in the dark place were calculated by utilizing the average limit of the random movements. The following facts may be seen in the table.

1. Regardless of the kinds of angles, at each given angle the value is invariably smaller than that in the dark place or of random movements. The fact shows that the worms from which the anterior 3-4 segments were removed exhibit the positive orientation.

2. Except the case at the given angle 0° the magnitude of the average or the positive occupied angle is smallest at a distance of 2 meters from the light source and either increased or decreased distance increases the magnitude of the average or the positive occupied angle, or decreases the magnitude of the average or the positively moved angle. Nearly the same change is seen even in the negative occupied and negatively moved angles. These facts seem to suggest that when the worms were put under the influence of the horizontal light of about 100 meter-candles (2 m. distance) they attain most easily the position parallel to the light rays, but the increased intensity of light more or less forces the worms to deviate from the direction toward the light and the decreased intensity less influences to cause the positive orientation till finally in the dark place the movements become random.

3. At the given angle 0°, in the limit of the horizontal light, the average angle is maximum at a distance of 2 meters from the light source and either decreased or increased distance decreases the magnitude of the average occupied angle, though an increased distance above 4 or more meters tends again to increase the magnitude.

To see easily the state of changes in the moved angles with the increasing magnitude of the given angle, Fig. 12 was plotted from the section of the moved angles in Table 33. In the figure the magnitude of the given angles is given on abscissa and the moved angles on ordinate. The angles upward from the abscissa passing 0° show the positively and those downward the negatively moved angles. The tracings of the average moved angles are denoted by the thicker lines, and those of the positively and negatively moved angles by the thinner lines. The tracings of the angles at a distance of 2 meters from the light source at the elevation angle 25° are denoted by the full lines, those at a distance of 1 meter from the horizontal light source by the broken lines, those at a distance of 2 meters by the one-dotted chain lines, those at 3 meters by the two-dotted chain lines, those at 4 meters by the three-dotted chain lines and those in the dark place by the dotted lines. Moreover the straight line obliquely drawn represents the tracing of the theoretically maximum magnitude of the positively moved angle at each given angle.

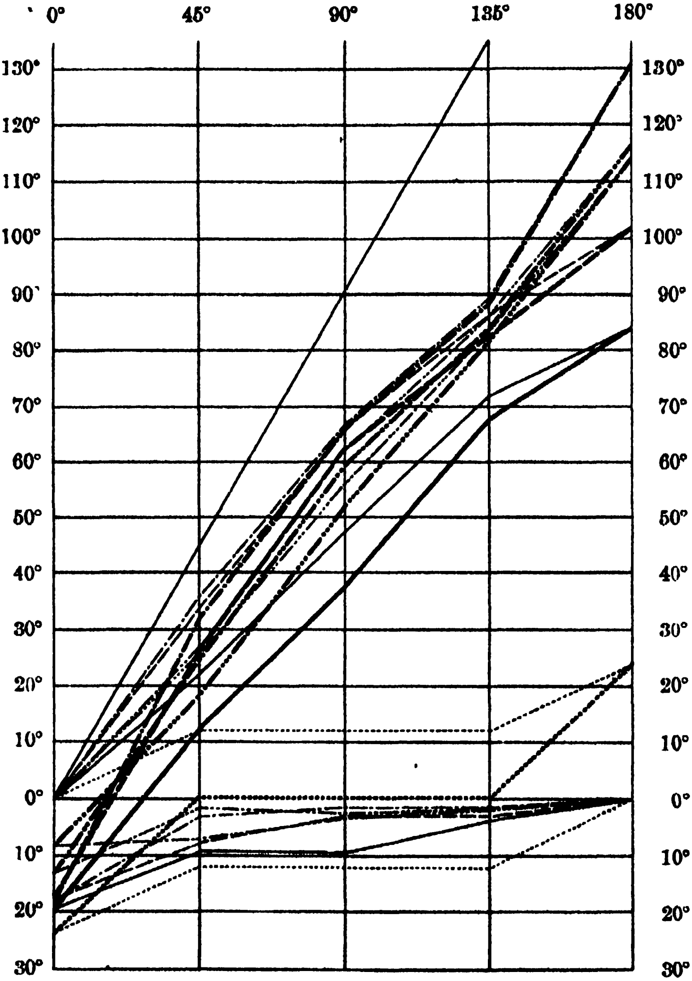
The following facts may be seen in Fig. 12.

1. All tracings of the average and positively moved angles show far larger angles than those of the average and positive random angles, though the former angles are smaller than the maximum moved angles. The difference between the maximum moved angle and the average or the positively moved angle becomes larger gradually with the increasing magnitude of the given angle.

2. The tracing of the average and positively moved angles at a distance of 2 meters from the elevated light source (full line) and at a distance of 1 meter from the horizontal light source (broken line) show a tendency of downward curvature while the remaining tracings show a tendency of upward curvature when the given angle increases from 135° to 180°. The fact seems to show that when the worms were exposed to a stronger light than nearly 100 meter-candles they crawl rather away from the light while under the influence of the light of nearly 100 meter-candles the worms exhibit fully the positively orienting movements, but when the intensity of the light further decreases it will not be sufficient to cause the strong positively orienting movements and consequently the worms begin to be less forced to orient toward the direction of the light and consequently some random movements appear to take place.

3. On the other hand, at the given angle 0°, if we assume that

Fig. 12.



the tracings are almost represented by straight lines which intersect each other at the given angle of small magnitude, a decrease of the magnitude of the average moved angle following an increase of the distance from the light source above 2 meters must take place, for if at the given angle 180° the terminal of a tracing moves downwards, then at the given angle 0° the terminal of the same tracing must move upwards because the line intersects as already stated with other lines at a point. When however the distance increases more a tracing such as was assumed in the above must become to resemble the tracing of the random movements in the dark place which can never be a straight line. We conclude therefore that when the worms were put toward the light, if the intensity of the light was strong the magnitude of the negatively moved angle is large because the worms show to some extent the negatively orienting nature, but if the intensity of the light decreases to some extent the moved angle becomes small because the worms make fully the positively orienting movements and if the intensity of the light decreases further the moved angle becomes large again because the worms are released from the influence of the light and thus make random movements.

CONCLUSIONS.

1. In the worms, from which the anterior 3–4 segments containing the brain were removed, the positive phototaxis is more pronounced than in the unoperated worms.

2. The worms, from which the anterior 3–4 segments were removed, show the positive orientation from the influence of light. It is ascertained by Hess (1924) that when the brain is removed in Lumbricus terrestris, the worm becomes to turn the anterior end of the body toward light.

3. The majority of the worms usually crawl forward while some crawl backward, exhibiting the positive orientation.

i. Even at the beginning of the movements the majority of the worms elongate the anterior end toward or the posterior end away from the light.

ii. Some worms make several preliminary movements before the definitive crawling takes place at the anterior or at the posterior end of the body either alternately or rarely synchronously, but the majority of the worms crawl backward directly without hesitation when they elongate the posterior end or forward when they elongate the anterior end.

iii. When the worms were placed under the influence of the horizontal light of about 100 meter-candles nearly half the number of the worms crawl either forward or backward with some hesitation, but under the influence of a light stronger or weaker than this the majority of the worms crawl forward with less hesitation.

iv. When the worms were exposed to a strong light or put toward the light they begin the posterior elongation, but if the light was of a comparatively low intensity, even when the worms were put toward the light, many worms crawl forward.

v. Even under the influence of a comparatively strong light when the worms were put at a given angle larger than 45°, they begin to crawl forward.

vi. Under the influence of a horizontal light of comparatively low intensity the greater the magnitude of the given angle, the easier is the forward crawling.

vii. In the dark place the worms always crawl forward and make random movements, the average limit of which is also ±12° at the fourth angle as in the unoperated worms.

4. Under the influence of a comparatively strong light some worms make winding movements while crawling either forward or backward.

5. To crawl forward or backward to attain a position parallel to the light rays is the same manifestation of the positive orientation of the worms.

i. When the worms were put toward the light the increased intensity of light forces the worms to deviate from the given direction.

ii. When the worms were put at the given angles 45°, 90°, 135° and 180° they exhibit most strongly the positive orientation under the influence of the horizontal light of nearly 100 meter-candles, but the increased intensity of the light forces the worms to deviate away from the light and the decreased intensity has less influence to cause the positive orientation and finally in the dark place the worms make random movements.

None of the above behavior is displayed by the worms from which the anterior segments were not removed. So it seems to me to be sure that when the brain is removed the ganglionic cells (?) which cause the positive orientation as well as the backward crawling are distributed in some place of the ventral nerve cord.

Chapter VII. The axially graded reactions of the worms against light.

It was tentatively concluded in Chapter VI that the ganglionic cells (?) causing either the positively orienting movements or the backward crawling movements are distributed not in the brain but in some portion of the ventral nerve cord. The object of the following experiments was to test the above hypothesis.

Experiments On Worms From Which Some Anterior Segments Were Removed.

For the purpose of investigation 7 experiments were carried on under the influence of a horizontal light of 100 meter-candles. In each experiment some anterior segments were removed from each worm just before the trial. When many segments were removed from the worms, most of them exhibited winding movements and consequently the angles in their tracks were not measurable except at the first progress, so in the present chapter only the changes in the first angle (Chapter I, also see Chapter VI) alone were studied. In most cases, however, even the track at the first progress was difficult to determine owing to successive winding movements, so the direction of crawl was marked as soon as each worm made the first progress and the measurement was made afterwards.

Experiment I. Only the prostomium was removed. 10 worms were tested at each given angle during the latter part of June.

Experiment II. The anterior 3–4 segments were removed. Here the results of the first angles from Experiment III in Chapter VI were utilized.

Experiment III. The anterior 10–11 segments were removed. 50 worms were tested at each given angle during the middle part of July.

Experiment IV. The preclitellar portion was removed. 50 worms were tested at each given angle during the middle part of August.

Experiment V. The anterior 32 segments were removed. 40 worms were tested at the given angle 90° during the middle part of August.

Experiment VI. The anterior 40 segments were removed. 50

worms were tested at each given angle during the latter part of August.

Experiment VII. The posterior half of the tail or post-clitellar portion was used. 40 worms were tested at the given angle 90° during the latter part of August.

In Table 34 the percentage values of those worms which began to elongate the posterior end, those which directly and those which definitively crawled backward are shown together. The following facts may be seen in the table.

TABLE 34.

	Portions removed / Given angles	Prostomium	3–4 segments	10–11 segments	Preclitellar portion	32 segments	40 segments	½ post-clitellar portion *left*
Percentage values of worms which began to move at the posterior end of the body.	0°	0	78	32	20		4	
	45°	0	38	22	12		6	
	90°	0	61	22	4	5	2	0
	135°	0	36	16	6		6	
	180°	0	68	8	0		0	
Percentage values of worms which directly crawled backward.	0°	0	70	10	4		0	
	45°	0	12	11	0		0	
	90°	0	42	6	2	0	0	0
	135°	0	20	2	0		0	
	180°	0	30	2	0		0	
Percentage values of worms which definitively crawled backward.	0°	0	78	14	4		0	
	45°	0	40	17	0		0	
	90°	0	59	10	2	0	0	0
	135°	0	42	4	0		0	
	180°	0	54	4	0		0	

1. When only the prostomium was removed the worms, in which

the brain is intact, show neither the posterior elongation nor the backward crawling, while when the anterior 3–4 segments were removed many worms, in which the brain is absent, show either the posterior elongation or the backward crawling. This fact may be taken to mean that the ganglionic cells (?) causing the posterior elongation are distributed in the ventral nerve cord, while the brain causes only the forward crawling and inhibits the posterior elongation.

2. At each given angle when the anterior 3-4 segments were removed the frequency of posterior elongation is maximum, and in association with the removal of a larger number of segments the frequency of posterior elongation decreases. When the anterior 32–40 segments were removed the backward crawling ceases, though sometimes the posterior elongation occurs, and finally in the worms in which half of the post-clitellar portion was left the posterior elongation never occurs. These facts seem to show that the ganglionic cells (?) causing the posterior elongation are distributed mainly in the anterior portion of the ventral nerve cord and in general the frequency of posterior elongation is proportional to the number of posterior segments left.

3. Even those worms which showed the posterior elongation, when some stimuli were applied (See Chapter VI), show also the anterior elongation or forward crawling. This fact shows that the ganglionic cells (?) causing the anterior elongation are distributed through the whole length of the ventral nerve cord.

In Table 35 the percentage values of the positively and the negatively phototactic worms are given together. The kinds of phototaxis of the worms were judged in the same way as in Chapter VI. The table reveals no regular changes of the percentage values.

The percentage values of individuals which showed the winding movements are given in Table 36. In the table it is seen that when the larger number of anterior segments were removed the frequency of winding movements increased. It was intimated in Chapter VI that the uneven structure or development of setae or the unevenly cut end of the body might be one of the factors which cause mechanically the winding movements. If the supposition were true the worms ought to wind toward only one side of the body, but actually we find that many worms could wind dextrally even when they crawled out toward the left side of the body and *vice versa*. This fact seems to show that the slight unevenness in setae or at the cut end of the body is less effective as the initiative cause of the winding movements. And as has already been mentioned in Chapter VI, if it can be granted that the ganglionic cells (?) causing the positive orientation are present in the ventral nerve cord, the worms ought to show the positive orientation irrespective of the modes of crawling. With this view in mind I have determined the first angles and the results are shown in Tables 37 and 38, both the cases of crawling forward and backward being united.

TABLE 35.

Given angles	Portions removed	Positive	Negative
0°	Prostomium	100	0
	3–4 segments	20	80
	10–11 segments	86	14
	Preclitellar portion	96	4
	40 segments	84	16
45°	Prostomium	60	40
	3–4 segments	62	38
	10–11 segments	83	17
	Preclitellar portion	92	8
	40 segments	66	34
90°	Prostomium	50	50
	3–4 segments	38	62
	10–11 segments	68	32
	Preclitellar portion	60	40
	32 segments	60	40
	40 segments	62	38
	½ post-clitellar portion *left*	62	38
135°	Prostomium	40	60
	3–4 segments	34	66
	10–11 segments	32	68
	Preclitellar portion	34	66
	40 segments	42	58
180°	Prostomium	0	100
	3–4 segments	46	54
	10–11 segments	4	96
	Preclitellar portion	2	98
	40 segments	0	100

In Table 37 the number of individuals distributed in each angular section are shown in percentage values. The following facts may be seen in the table.

1. At the given angles 45°, 90° and 135° the number of individuals which make occupied angles smaller than the corresponding given angle is greater when the anterior 3–4 segments were removed than when only the prostomium was removed. At the given angle 0° the number of individuals which occupied angles smaller than 45° is greater when the anterior 3–4 segments were removed than when only the prostomium was removed. And at the given angle 180° when only the prostomium was

TABLE 36.

Portions removed / Given angles	Prostomium	3–4 segments	10–11 segments	Preclitellar portion	32 segments	40 segments	½ post-clitellar portion *left*
0°	0	0	16	60		82	
45°	0	0	18	72		88	
90°	0	1	32	60	80	80	90
135°	0	4	16	68		84	
180°	0	10	24	82		88	

removed no worms occupied angles smaller than 135°, but when the anterior 3–4 segments were removed nearly half the number of the worms occupied angles smaller than 135°. These facts show that the worms exhibit a stronger positive orientation when the anterior 3–4 segments were removed than when only the prostomium was removed.

2. At the given angles 0°, 45° and 90° when a larger number of segments were removed the number of positively orienting individuals tends to decrease.

3. At the given angle 135° nearly the same phenomena as stated in the above are also seen, but in this case the number of positively orienting individuals becomes greater when the anterior 40 segments were removed than when the preclitellar portion was removed. At the given angle 180° the number of individuals which made occupied angles smaller than 135° is greater when 40 segments were removed than when the preclitellar portion was removed which in turn is greater than when 10–11 segments were removed. These phenomena are probably due to the tight winding movements which happen very often when many anterior segments are removed.

In Table 38 the changes in the magnitude of angles are shown. The following facts may be seen in the table.

1. When the prostomium was removed, the average occupied angle at each of the given angles 45°, 90° and 135° showed an intimate approximation to the magnitude of the corresponding given angle. This fact seems to suggest that when only the prostomium is removed the phototaxis or orientation becomes obscure and consequently the

TABLE 37.

Given angles	Portions removed	0°-45°	46°-90°	91°-135°	136°-180°
0°	Prostomium	80	20	0	0
	3-4 segments	94	6	0	0
	10-11 segments	88	12	0	0
	Preclitellar portion	76	24	0	0
	40 segments	54	34	12	0
45°	Prostomium	60	40	0	0
	3-4 segments	88	12	0	0
	10-11 segments	80	20	0	0
	Preclitellar portion	56	38	6	0
	40 segments	40	26	26	8
90°	Prostomium	0	80	20	0
	3-4 segments	30	64	3	3
	10-11 segments	26	54	18	2
	Preclitellar portion	36	26	30	8
	32 segments	23	38	22	17
	40 segments	26	38	14	22
	½ post-clitellar portion *left*	42	20	8	30
135°	Prostomium	0	0	70	30
	3-4 segments	12	28	46	14
	10-11 segments	10	24	42	24
	Preclitellar portion	16	20	18	46
	40 segments	2	42	24	32
180°	Prostomium	0	0	0	100
	3-4 segments	0	16	36	48
	10-11 segments	4	0	28	68
	Preclitellar portion	2	0	36	62
	40 segments	0	2	44	54

worms show movements which are nearly the same as random movements.

2. When the anterior 3–4 segments were removed the occupied angles became smaller than when only the prostomium was removed, and this fact is especially true in the average and positive occupied angles. This fact shows that when the anterior 3–4 segments are removed, the negatively orienting movements almost disappear and the positively orienting movements begin to take their place.

3. When the anterior 10–11 segments were removed, the occupied angles became larger or the degree of positive orientation became weaker than when the anterior 3–4 segments were removed.

4. When the preclitellar portion was removed, the occupied angles became larger than when the anterior 10–11 segments were removed, though the positive occupied angles at the given angles 90°, 135° and 180° showed a slight decrease. The decrease of the positive occupied angles and the increase of the negative occupied angles in part are, in my opinion, due to the increased frequency of winding movements (See Table 36), for when the worms perform winding movements they generally make larger moved angles either toward or away from the light. So this fact appears to show that when the preclitellar portion is removed, the degree of negative orientation grows stronger and consequently, as is shown in the average occupied angles, the positive orientation becomes less pronounced in comparison with the case of the removal of 10–11 segments.

5. When the anterior 40 segments were removed, in general the occupied angles became larger or the degree of negative orientation became stronger than when the preclitellar portion was removed.

6. When the posterior half of the post-clitellar portion was left, the positive occupied angle became smaller while the negative occupied angle became larger than when the anterior 40 segments were removed. These phenomena are due to the increased frequency of the winding movements, the winding of which becomes tighter as has been stated already when many anterior segments were removed. And from the values of the occupied angles we may conclude that the positive and the negative orientations are nearly equally exhibited at the given angle 90° when a larger number of anterior segments than 32 are removed, though the positive orientation is a little more pro-

TABLE 38.

	Portions removed	Average angles					Positive angles					Negative angles				
		0°	45°	90°	135°	180°	0°	45°	90°	135°	180°	0°	45°	90°	135°	180°
		in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.	in degr.
Occupied angles	Prostomium	31.70	42.90	89.50	132.50	169.00	0.00	38.20	85.80	128.60	169.00	31.70	49.70	93.70	138.90	180.00
	3–4 segments	14.74	28.00	58.84	95.86	127.30	0.00	26.82	56.46	93.76	127.30	14.74	46.18	92.38	137.10	180.00
	10–11 segments	21.08	33.17	72.88	105.78	145.50	0.00	28.72	69.10	102.50	145.50	21.08	49.45	93.78	138.28	180.00
	Preclitellar portion	35.06	44.12	74.36	110.78	143.10	0.00	30.54	61.20	100.54	143.10	35.06	58.58	103.16	145.24	180.00
	32 segments			82.28					63.18					109.10		
	40 segments	52.60	65.42	83.54	112.14	141.52	0.00	34.38	63.78	102.92	141.52	52.60	76.04	109.76	144.22	180.00
	½ post-clitellar portion *left*			81.08					56.58					114.50		
Moved angles	Prostomium	-31.70	2.10	0.50	2.50	11.00	0.00	6.80	4.20	6.40	11.00	-31.70	-4.70	-3.70	-3.90	0.00
	3–4 segments	-14.74	17.00	31.16	39.14	52.70	0.00	18.18	33.54	41.24	52.70	-14.74	-1.18	-2.38	-2.10	0.00
	10–11 segments	-21.08	11.83	17.12	28.22	34.50	0.00	16.28	20.90	32.50	34.50	-21.08	-4.45	-3.78	-3.28	0.00
	Preclitellar portion	-35.06	0.88	15.64	24.22	36.90	0.00	14.46	28.80	34.46	36.90	-35.06	-13.58	-13.16	-10.24	0.00
	32 segments			7.72					26.82					-19.10		
	40 segments	-52.60	-20.42	6.46	22.86	38.48	0.00	10.62	26.22	32.08	38.48	-52.60	-31.04	-19.76	-9.22	0.00
	½ post-clitellar portion *left*			8.92					33.42					-24.50		

nounced than the negative orientation.

7. The removal of anterior segments decreased the magnitude of the negatively moved angle and increased that of the positively moved angle with the increasing magnitude of the given angle, and the removal of a larger number of anterior segments tended to make a quicker increase of the magnitude of the negatively moved angle at a smaller given angle than at a larger given angle. These facts show that the smaller given angle causes the positive orientation to be less and the negative orientation to be more pronounced. In fact when the preclitellar portion was removed, at the given angle 45° the average moved angle approached to 0°, and when the anterior 40 segments were removed, at the given angle 45° the average moved angle became negative, and at a given angle smaller than 90° it approached 0°, while at the larger given angles the average moved angles were still positive.

8. At the given angle 180° the angular values apparently show that the removal of a larger number of segments than 11 caused the positive orientation to be more pronounced. But the changes are only due to the increased frequency of winding movements which become tighter when many anterior segments are removed and consequently the changes do not mean the positive orientation which is more prononnced. Similarly the increase of the negative angular values at the given angle 0° partially may be considered as the result of the winding movements.

According to the facts from Tables 37 and 38, when the anterior 3–4 segments which contain the brain are removed, the degree of positive orientation is strongest, and when a larger number of anterior segments are removed the positive orientation becomes less and the negative orientation more pronounced. Furthermore when the preclitellar protion is removed, the movements become random at the given angle 45° and similarly when the anterior 32 or more segments are removed, the movements also become random at a given angle smaller than 90°. These facts seem to show that in the anteriormost portion of the ventral nerve cord the ganglionic cells (?) causing the positive orientation can influence more strongly than the ganglionic cells (?) causing the negative orientation, but posteriorly to the clitellum both cells (?) are capable of reacting equally but respond differentially to the light when the worms are placed at different given angles.

EXPERIMENTS ON WORMS FROM WHICH SOME POSTERIOR SEGMENTS WERE REMOVED.

For the purpose of investigation 3 experiments were carried on under the influence of a horizontal light of 100 meter-candles. In each experiment some posterior segments were removed from each worm just before the trial. When an anterior piece of a few segments, viz. 3–4 or 10–11, was tested, in most cases it could not show the proper orienting movements owing probably to imperfection in the locomotive organs. In the following pages the results from the first angles alone are stated for the purpose of comparison with the results in the preceding experiments.

Experiment I. The preclitellar portion was used. 40 worms were tested at the given angle 90° during the middle part of August.

Experiment II. The anterior 32 segments were used. 40 worms were tested at the given angle 90° during the latter part of August.

Experiment III. The anterior 40 segments were used. 50 worms were tested at the given angle 90° during the latter part of August.

In each experiment the anterior pieces invariably crawled forward, though in a few a tendency toward backward crawling was detectable. So in the present experiments the orientations of the pieces decisively coincide with the phototaxes.

The results of measuring the angles are shown in Tables 39 and 40.

TABLE 39.

Portions used	0°–45°	46°–90°	91°–135°	136°–180°
Preclitellar portion	2.5	12.5	37.5	47.5
32 segments	2.5	10.0	50.0	37.5
40 segments	0.0	14.0	52.0	34.0

In Table 39 the frequency distribution of the worms in 4 angular sections, viz. 0°–45°, 46°–90°, 91°–135° and 136°–180°, is shown in percentage values. The table reveals that the percentage value becomes smaller in the section 136°-180°, while it becomes greater in the section 91°-135° with the increasing number of segments. This fact shows that a larger number of segments tends to decrease the frequency of stronger orienting or phototactic movements.

TABLE 40.

	Portions used	Average angles in degrees	Positive angles in degrees	Negative angles in degrees
Occupied angles	Preclitellar portion	127.60	87.38	130.22
	32 segments	127.08	88.05	129.03
	40 segments	126.24	89.88	126.36
Moved angles	Preclitellar portion	-37.60	2.62	-40.22
	32 segments	-37.08	1.95	-39.03
	40 segments	-36.24	0.12	-36.36

In Table 40 the changes in the magnitude of the angles are shown. According to the table the average and the negative angles show that the negative orientation or phototaxis tends to be less pronounced with the increasing number of segments, and this coincides with the fact from Table 39.

EXPERIMENTS ON WORMS FROM WHICH SOME ANTERIOR AND SOME POSTERIOR SEGMENTS WERE REMOVED.

For the purpose of investigation 2 experiments were carried on under the influence of a horizontal light of 100 meter-candles.

Experiment I.

In the experiment the anterior 3-4 segments and the posterior half of the post-clitellar portion were removed from each worm just before the trial. 50 worms were tested at the given angle 90° during the early part of August.

Frequency of the first movement at the anterior end of the

body. 48%
Frequency of the direct forward crawling. 44%
Frequency of the definitive forward crawling. 92%
Frequency of the first movement at the posterior end of the body. 42%
Frequency of the direct backward crawling. 2%
Frequency of the definitive backward crawling. 8%
Frequency of the synchronous movements at both ends of the body. 10%
Frequency of the winding movement. 8%
Number of the positively phototactic worms. 70%
Number of the negatively phototactic worms. 30%

The results of measuring the angles are as follows:

The frequency distribution which determines the kinds of orientation of the worms is shown below in percentage values.

0°-45°	46°-90°	91°-135°	136°-180°
26	56	16	2

The angular values which determine the degrees of orientation of the worms are shown below.

	Occupied angles	Moved angles
Average	70.10°	19.90°
Positive	65.02°	24.98°
Negative	95.08°	-5.08°

Experiment II.

In this experiment the preclitellar portion and the posterior half of the post-clitellar portion were removed from each worm just before the trial. 40 worms were tested at the given angle 90° during the early part of August.

All worms progressed forward, 90% of them making winding movements.

The results of measuring the angles are as follows:

The frequency distribution which determines the kinds of orientation of the worms is shown below in percentage values.

0°-45°	46°-90°	91°-135°	136°-180°
25.0	37.5	10.0	27.5

The angular values which determine the degrees of orientation of the worms are shown below.

	Occupied angles	Moved angles
Average	83.53°	6.47°
Positive	65.63°	24.37°
Negative	107.90°	-17.90°

COMPARISON OF THE RESULTS FROM THE PRECEDING EXPERIMENTS.

In the following pages the results from the first angles at the given angle 90° alone are stated.

1. Comparison of the results from the experiments in connection with severance just in front of the clitellum.

When the unoperated worms were tested they showed the average moved angle -23.85° and when the anterior pieces were tested they showed the average moved angle -37.60°. Thus the anterior pieces showed a negative orientation stronger than the unoperated worms, the strengthened degree of which may be expressed by -13.75° (-37.60°+23.85°).

When the posterior pieces were tested they showed a positive orientation which may be expressed by the average moved angle 15.64°.

Thus the angular value 15.64° which expresses the positivity of the posterior pieces shows an intimate approximation to the angular value -13.75° which expresses the strengthened negativity of the anterior pieces above the negativity of the unoperated worms.

2. Comparison of the results from the experiments in connection with severance just behind the clitellum.

When the unoperated worms were tested they showed the average moved angle -23.85° and when the anterior pieces were tested they showed the average moved angle -37.08°. Thus the anterior pieces showed a negative orientation stronger than the unoperated worms, the strengthened degree of which may be expressed by -13.23° (-37.08°+23.85°).

When the posterior pieces were tested they showed a positive orientation which may be expressed by the average moved angle 7.72°.

3. Comparison of the results from the experiments in connection

with severance at the 40th segment.

When the unoperated worms were tested they showed the average moved angle -23.85° and when the anterior pieces were tested they showed the average moved angle -36.24°. Thus the anterior pieces showed a negative orientation stronger than the unoperated worms, the strengthened degree of which may be expressed by -12.39° (-36.24°+23.85°).

When the posterior pieces were tested they showed a positive orientation which may be expressed by the average moved angle 6.46°.

4. Comparison of the results from the experiments in connection with severances at the 3rd or 4th segment as well as at the middle of the post-clitellar portion.

The anterior pieces could not show proper orienting movements.

When the worms, from which the anterior 3-4 segments were removed, were tested they showed the average moved angle 31.16° and when the middle pieces were tested they showed the average moved angle 19.90°. Thus the middle pieces showed a positive orientation weaker than the worms from which the anterior 3-4 segments were removed, the weakened degree of which may be expressed by -11.26° (19.90°-31.16°).

When the posterior pieces were tested they showed a positive orientation which may be expressed by the average moved angle 8.92°.

Thus the angular value 8.92° which expresses the positivity of the posterior pieces shows a near identity to the angular value -11.26°, which expresses the weakened positivity of the middle pieces below the positivity of the worms from which the anterior 3-4 segments were removed.

The combined positivity of the middle and posterior pieces may be expressed by 28.82° (19.90°+8.92°), and this value shows a near identity to the value 31.16° which expresses the positivity of the worms from which the anterior 3-4 segments were removed.

5. Comparison of the results from the experiments in connection with severances just in front of the clitellum as well as at the middle of the post-clitellar portion.

When the worms, from which the preclitellar portion was removed, were tested they showed the average moved angle 15.64° and when the middle pieces were tested they showed the average moved angle 6.47°. Thus the middle pieces showed a positive orientation weaker than the worms from which the preclitellar portion was removed, the weakened degree of which may be expressed by -9.17° (6.47°-15.64°).

When the posterior pieces were tested they showed a positive orientation which may be expressed by the average moved angle 8.92°.

Thus the angular value 8.92° which expresses the positivity of the posterior pieces shows an intimate approximation to the angular value -9.17° which expresses the weakened positivity of the middle pieces below the positivity of the worms from which the preclitellar portion was removed.

When the anterior pieces were tested they showed a negative orientation stronger than the unoperated worms, the strengthened degree of which may be expressed by -13.75°.

The combined positivity of the middle and posterior pieces may be expressed by 15.39° (6.47°+8.92°), and this value is approximately identical with the value 15.64° which expresses the positivity of the worms from which the preclitellar portion was removed.

Thus the value -13.75° which expresses the strengthened negativity of the anterior pieces above the negativity of the unoperated worms shows a close approximation to the value 15.39° which expresses the combined positivity of the middle and posterior pieces or the positivity of the worms from which the preclitellar portion was removed.

From the above relations, though some discrepancies are found, we may infer the following general relations:

1) When the worms are severed transversely a smaller number of the anterior segments containing the brain strengthen the degree of negative orientation and a larger number of the posterior segments without the brain strengthen the degree of positive orientation.

2) When the worms are severed transversely a larger number of the anterior segments containing the brain weaken the degree of negative orientation and a smaller number of the posterior segments without the brain weaken the degree of positive orientation.

3) When degrees of the orientation are expressed by absolute angular values, the strengthened or weakened degree of the negative orientation of the anterior piece balances with the strengthened or

weakened degree of the positive orientation of the posterior piece.

4) When the posterior piece is divided, the degree of the positive orientation of the whole piece balances with the sum of the degrees of the positive orientations of the divided pieces.

CONCLUSIONS.

1. The worms show axially graded orienting reactions in movements against light.

i. When only the prostomium is removed, the worms in which the brain is intact crawl forward and show movements nearly the same as random movements.

ii. When some anterior segments containing the brain are removed, the worms show several modes of movements.

When the anterior 3–4 segments are removed, the worms can crawl either forward or backward and show the positive orientation.

The removal of a larger number of the anterior segments shows a less pronounced positive orientation, a more pronounced negative orientation, a decreased frequency of posterior elongation, and an increased frequency of winding movements.

When the anterior 32 or more segments are removed, the backward crawling ceases, and when the anterior 50 or more segments are removed (= posterior half of the post-clitellar portion left), even the posterior elongation ceases.

When the preclitellar portion is removed, the worms show random movements at the given angle 45°, and when the anterior 32 or more segments are removed, the worms show negative orientation at the given angle 45° and random orientation at a given angle smaller than 90°, while at a larger given angle they show positive orientation.

iii. When some posterior segments are removed, the worms crawl forward and the removal of a larger number of the posterior segments causes a more pronounced negative orientation.

2. From the above different movements, we can verify the following hypotheses.

i. The brain causes negative orientation and inhibits posterior elongation as well as backward crawling.

ii. In the ventral nerve cord we can distinguish 4 kinds of ganglionic cells (?) of different functions against light:

The ganglionic cells (?) causing anterior elongation and forward crawling. These cells (?) appear to be distributed through the whole length of the ventral nerve cord.

The ganglionic cells (?) causing posterior elongation and backward crawling. These cells (?) appear to be distributed densely in the anterior portion of the ventral nerve cord.

The ganglionic cells (?) causing negative orientation. These cells (?) appear to be distributed through the whole length of the ventral nerve cord, but the distribution is less in the anteriormost portion.

The ganglionic cells (?) causing positive orientation. These cells (?) appear to be distributed through the whole length of the ventral nerve cord, but the distribution is densest in the anteriormost portion of the ventral nerve cord.

3. When the worms are divided into 2 pieces, anterior and posterior, the strengthened or weakened degree of the negative orientation of the anterior pieces balances with the strengthened or weakened degree of the positive orientation of the posterior pieces.

Chapter VIII. The influence of light upon regenerating worms.

The object of the present experiments was to see what kind of orientation or phototaxis would appear in the worms which showed a slight regeneration at the cut end.

EXPERIMENT I.

The experiment was carried on during the latter part of June. The worms in which the anterior 3–4 segments were removed were kept in a dark place and at intervals of from 67 to 87 hours after operation they were tested by placing them at the given angles 0°, 45°, 90°, 135° and 180° under the influence of a horizontal light of 100 meter-candles. 50 worms were tested at each given angle. As the control of the present experiment, those data, which were given in Chapter VI and in which the movement of the worms was tested immediately after the removal of the anterior 3–4 segments, were utilized.

TABLE 41.

	Given angles	First movement at tail end	Direct forward crawling	Crawling with preliminary movement	Direct backward crawling	Definitive forward crawling	Positive phototaxis	Negative phototaxis
Results from the experiments made soon after operation.	0°	78	12	18	70	22	22	78
	45°	38	34	54	12	60	62	38
	90°	61	26	32	42	41	43	56
	135°	36	32	48	20	58	64	32
	180°	68	22	48	30	46	46	44
	Average	56	25	40	35	45	47	50
Results from the experiments made after 67–87 hours.	0°	70	28	10	62	26	34	64
	45°	62	38	26	36	64	50	44
	90°	64	28	24	48	42	42	52
	135°	34	56	28	16	80	30	70
	180°	26	64	22	14	84	30	68
	Average	51	43	22	35	61	37	60

It may be seen in Table 41 that in general the frequencies of posterior elongation and of direct backward crawling are nearly equal in either case of tests, but the frequencies of direct and of definitive forward crawling are greater in the regenerating worms than in the worms just operated upon. In the same table the numbers of the positively and the negatively phototactic worms are given excluding the number of winding worms, and these values show that in general while the operated worms are regenerating the degree of negative phototaxis of them grows stronger. In general the forward crawling worms show positive phototaxis and the backward crawling worms show negative phototaxis.

The results of measuring the fourth angles (See Chapter I) are shown in Tables 42 and 43, both cases of forward and backward crawlings being united.

TABLE 42.

	Given angles	Distribution of worms				Winding	Orientation		
		0°–45°	46°–90°	91°–135°	136°–180°		Positive	Indifferent	Negative
Results from the experiments made soon after operation.	0°	88	10	2	0	0	74		26
	45°	94	4	0	2	0	86	10	4
	90°	81	12	5	1	1	89	2	8
	135°	52	26	14	4	4	88	4	4
	180°	52	16	14	8	10	88	2	
Results from the experiments made after 67–87 hours.	0°	88	6	0	4	2	82		16
	45°	62	24	6	2	6	58	8	28
	90°	40	32	10	12	6	60	18	16
	135°	8	34	26	32	0	64	10	26
	180°	26	6	18	48	2	66	32	

In Table 42 the frequency distribution of the worms is given in percentage values. In the table the distribution of worms shows that in general the regenerating worms made occupied angles larger than those which were made by the worms just operated upon, and in the part of the orientation it is seen that in general the degree of positive orientation was stronger in the worms just operated upon than in the regenerating worms. These facts show that in the regenerating worms the degree of negative orientation becomes increased in comparison with the worms just operated upon.

The angular values in Table 43 show that in general the degree of positive orientation is weaker while the degree of negative orientation is stronger in the regenerating worms than in those just operated upon.

To study the orienting difference between the forward and backward crawling worms Table 44 was made. In the table the following facts may be seen.

1. The number of tracks of the worms which crawled forward is greater in the regenerating worms than in those just operated upon.
2. The degree of positive orientation of the forward crawling

TABLE 43.

	Given angles	Results from the experiments made soon after operation		Results from the experiments made after 67–87 hours.	
		Occupied angles	Moved angles	Occupied angles	Moved angles
		in degrees	in degrees	in degrees	in degrees
Average angles	0°	18.06	-18.06	17.96	-17.96
	45°	12.74	32.26	37.04	7.96
	90°	24.32	65.68	66.50	23.50
	135°	46.79	88.21	106.76	28.24
	180°	49.47	130.53	112.51	67.49
Positive angles	0°	0.00	0.00	0.00	0.00
	45°	9.38	35.62	24.40	20.60
	90°	23.06	66.94	54.17	35.83
	135°	45.46	89.54	98.80	36.20
	180°	49.47	130.53	112.51	67.49
Negative angles	0°	18.06	-18.06	17.96	-17.96
	45°	48.36	-3.36	57.64	-12.64
	90°	91.26	-1.26	102.33	-12.33
	135°	136.33	-1.33	142.96	-7.96
	180°	180.00	0.00	180.00	0.00

worms becomes weakened while the worms are regenerating.

3. However in the backward crawling worms the positive orientation is nearly equally pronounced in either case tested immediately after operation or after 67–87 hours have elapsed. This fact is probably due to the smaller degree of regeneration at the cut end.

From Tables 41–44 we may conclude that even in the extent of the present degree of regeneration, the worms from which the anterior 3–4 segments were removed are capable 1) to crawl forward though posterior elongation and direct backward crawling are still frequent, and 2) to show a more pronounced negative orientation in comparison with the case tested immediately after operation.

TABLE 44.

	Given angles	Results from the experiments made soon after operation				Results from the experiments made after 67-87 hours.			
		Number of tracks	Number of winding tracks	Average occupied angles	Average moved angles	Number of tracks	Number of winding tracks	Average occupied angles	Average moved angles
		in %	in %	in degr.	in degr.	in %	in %	in degr.	in degr.
Results from the tracks crawled forward.	0°	22	0	12.45	-12.45	36	2	21.94	-21.94
	45°	60	0	6.93	38.07	64	6	50.76	-5.76
	90°	41	1	17.08	72.92	42	2	76.05	13.95
	135°	58	4	29.37	105.63	80	0	117.95	17.05
	180°	46	0	42.78	137.22	84	0	119.50	60.50
Results from the tracks crawled backward.	0°	78	0	19.64	-19.64	64	0	15.84	-15.84
	45°	40	0	21.45	23.55	36	0	14.94	30.06
	90°	59	0	29.24	60.76	58	4	59.07	30.93
	135°	42	0	69.19	65.81	20	0	62.00	73.00
	180°	54	10	57.50	122.50	16	2	70.57	109.43

EXPERIMENT II.

The experiment was carried on during the middle part of July. The worms in which the preclitellar portion was removed were kept in a dark place and at intervals of from 90 to 140 hours after operation they were tested by placing them at the given angles 0°, 45°, 90°, 135° and 180° under the influence of the horizontal light of 100 meter-candles. As the control of the present experiment, those data, which were given in Chapter VII and in which the movement of the worms was tested immediately after the removal of the preclitellar portion, were utilized.

In Table 45 it may be seen that the frequencies of posterior elongation and of backward crawling become greater and the frequency of winding movements becomes smaller in the regenerating worms than in those just operated upon, and that in general while the operated worms are regenerating the degree of negative phototaxis of them

TABLE 45.

	Given angles	First movement at tail end	Direct forward crawling	Crawling with preliminary movement	Direct backward crawling	Definitive backward crawling	Winding movements	Positive phototaxis	Negative phototaxis
Results from the experiments made soon after operation	0°	20	80	16	4	4	60	96	4
	45°	12	84	16	0	0	72	92	8
	90°	4	94	4	2	2	60	60	40
	135°	6	90	10	0	0	68	34	66
	180°	0	98	2	0	0	82	2	98
	Average	8.4	89.2	9.6	1.2	1.2	68.4	56.8	43.2
Results from the experiments made after 90–140 hours.	0°	44	56	26	18	20	32	76	24
	45°	28	72	20	8	8	20	82	18
	90°	26	74	18	8	10	30	52	48
	135°	18	82	12	6	6	28	18	82
	180°	12	88	10	2	2	22	2	98
	Average	25.6	74.4	17.2	8.4	9.2	26.4	46.0	54.0

becoms stronger. In the present experiment also the forward crawling worms showed in general positive phototaxis and the backward crawling worms showed negative phototaxis.

The results of measuring the first angles (See Chapter I and Chapter VII) are shown in Tables 46 and 47.

In Table 46 the frequency distribution of the worms is given in percentage values. In the table it is seen that in comparison with those just operated upon the regenerating worms tend to make smaller occupied angles at the given angles 0° and larger occupied angles at the given angles 90°, 135° and 180°. Moreover it is seen in the regenerating worms that at the given angles 90° and 135° the number of individuals which made occupied angles smaller than the corresponding given angle is nearly equal to that which made occupied angles larger than the corresponding given angle, and that the worms nearly equally deviate from the given direction when they were placed either

TABLE 46.

	Given angles	0°-45°	46°-90°	91°-135°	136°-180°
Results from the experiments made soon after operation	0°	76	24	0	0
	45°	56	38	6	0
	90°	36	26	30	8
	135°	16	20	18	46
	180°	2	0	36	62
Results from the experiments made after 90–140 hours.	0°	80	16	4	0
	45°	62	28	10	0
	90°	16	38	32	14
	135°	0	12	38	50
	180°	2	2	20	76

at the given angle 0° or 180°. These facts seem to show that while the worms are regenerating for a time the orientation becomes less pronounced and consequently the worms show random movements.

The changes in the angular values are shown in Table 47. The following facts may be seen in the table.

1. The negative occupied angles are nearly equal or the negative orientation is nearly equally pronounced in either case tested immediately after operation or after 90–140 hours have elapsed.

2. The positive occupied angles become smaller or the positive orientation becomes less pronounced in the regenerating worms than in those just operated upon.

3. In the regenerating worms the average moved angle nearly approaches to 0° at the given angles 45°, 90° and 135°, and the positively moved angle at the given angle 180° is nearly equal to the negatively moved angle at the given angle 0°. These facts suggest that the regenerating worms in the present experiment showed random movements. The larger value of the moved angles in comparison with the value in the worms unoperated upon is due to the larger angles caused by winding movements.

TABLE 47.

	Given angles	Results from the experiments made soon after operation.		Results from the experiments made after 90–140 hours.	
		Occupied angles	Moved angles	Occupied angles	Moved angles
		in degrees	in degrees	in degrees	in degrees
Average angles	0°	35.06	-35.06	31.56	-31.56
	45°	44.14	0.88	46.54	-1.54
	90°	74.36	15.64	86.22	3.78
	135°	110.78	24.22	134.46	0.54
	180°	143.10	36.90	149.78	30.22
Positive angles	0°	0.00	0.00	0.00	0.00
	45°	30.54	14.46	33.68	11.32
	90°	61.20	28.80	70.32	19.68
	135°	100.54	34.46	123.54	11.46
	180°	143.10	36.90	149.78	30.22
Negative angles	0°	35.06	-35.06	31.56	-31.56
	45°	58.58	-13.58	57.86	-12.86
	90°	103.16	-13.16	105.90	-15.90
	135°	145.24	-10.24	145.92	-10.92
	180°	180.00	0.00	180.00	0.00

From Tables 45–47 we may conclude as follows.

1. Even in the extent of the present degree of regeneration the worms from which the preclitellar portion was removed show more frequent backward crawling and less frequent winding movements in comparison with those just operated upon.

2. While the worms are regenerating at first the positive orientation becomes less pronounced and then the negative orientation becomes more pronounced.

3. While the worms are regenerating they show random movements for a time.

GENERAL REMARKS.

Even in the early stage of regeneration, the worms from which the anterior 3–4 segments were removed showed a less pronounced positive orientation and a more pronounced negative orientation. This movement of the worms must be related to the functioning of the regenerating brain, because as has been found in Chapters VI and VII no nervous system other than the brain can produce such a negative orientation.

The regenerating worms from which the preclitellar portion was removed showed less pronounced positive orientation and nearly equally pronounced negative orientation in comparison with those just operated upon. This movement of the worms just stated suggest that when the preclitellar portion was removed the nervous portion or brain which causes more a pronounced negative orientation is very slightly formed in such an early stage of regeneration.

From the above statements the following relations may be noticed.

1. When the smaller number of anterior segments were removed the brain regenerates more quickly than when the larger number of anterior segments were removed.

2. The degree of orientation of the worms is determined by the antagonistic relations between the functioning of the regenerating brain and that of the regenerating ventral nerve cord.

3. If in the positively orienting worms the positive orientation becomes less pronounced and the negative orientation becomes more pronounced, the worms ought to show random movements so long as both degrees balance each other.

We may infer further the following relations in the same hypothesis as is stated in the above.

Even in the early stage of regeneration when the anterior 3–4 segments were removed the worms tend to show an increased frequency of forward crawling because the quickly regenerating brain functions to cause forward crawling. But when the preclitellar portion was removed the regenerating worms tend to show an increasing frequency of backward crawling because the regenerating ventral nerve cord functions to cause backward crawling and the slightly regenerating brain does not function to inhibit backward crawling.

CONCLUSIONS.

1. In the early stage of regeneration, in the worms from which the anterior 3–4 segments were removed, the frequency of negative phototaxis becomes larger, because the degree of negative phototaxis becomes stronger in the forward crawling worms, and even in the worms from which the preclitellar portion was removed, the frequency of negative phototaxis also becomes larger, because the frequency of backward crawling away from the light becomes larger.
2. In the regenerating worms the frequency of winding movements becomes smaller and the positive orientation becomes less pronounced than in the worms just operated upon.
3. The function of the brain recovers inversely proportional to the number of anterior segments which were removed.
4. The phototactic reactions, orienting movements and forward or backward crawling, are determined by the relative dominance of the antagonistic functioning of the regenerating anterior portions of the nervous system, that is of the brain and of the ventral nerve cord.
5. Consequently the regenerating worms show random movements so long as the degrees of antagonistic orientations of the brain and of the ventral nerve cord balance each other.

Chapter IX. The phototactic antagonism between the brain and the ventral nerve cord. General conclusions.

The unoperated worms usually show negative phototaxis, but sometimes the worms become positively phototactic for a time and then show random movements either when the worms were exposed to stronger light or fatigued (Chapters I, III and IV). Moreover the worms from which the prostomium was removed show also random movements when they are tested immediately after operation (Chapter VII).

In testing the worms operated upon, we have learned that the brain causes negative orientation and forward crawling (that is negative phototaxis), while the ventral nerve cord causes positive orientation (Chapters VI and VII), and whether the orientation of the operated worms will become positive or negative is determined by the functioning of the nervous system (Chapters VII and VIII). It follows then that according to the degree of functioning of the brain, even the unoperated worms may show random movements or positive orientation by the functioning of the ventral nerve cord. It is the purpose of the present experiment to ascertain further in regard to this antagonistic relation just stated.

The experiment was carried on during the earlier part of August to see what kind of phototaxis would appear in the worms which were illuminated from the ventral side of the body.

For the purpose of investigation a glass plate was used its frontal margin being lifted its surface making an inclination of 15° against the horizontal light from an electric lamp of 500 watts at a distance of 2 meters. The frontal margin of the glass plate was shielded by a piece of wood to prevent the light rays from coming over the glass plate. The worms unoperated upon were tested on moistened paper which was spread over the upper surface of the glass plate at the given angles 0°, 45°, 90°, 135° and 180°. Thus the intensity of the light would be graded with the increasing distance from the light source, and though its degree was not determined, I may mention that in this case the intensity of the light inside the paper was sufficiently strong to read a book. 50 worms were tested at each given angle.

No cases of posterior elongation were observed in the experiment.

The results of measuring the fourth angles (See Chapter I) are given in Tables 48 and 49.

TABLE 48.

Given angles	0°-45°	46°-90°	91°-135°	136°-180°
0°	72	24	2	2
45°	46	30	16	8
90°	12	38	38	12
135°	4	14	30	52
180°	2	2	24	72

In Table 48 the frequency distribution of the worms is given in percentage values. It may be seen in the table that at the given angles 45°, 90° and 135° the number of individuals which made occupied angles smaller than the corresponding given angle is nearly equal to the number of individuals which made occupied angles larger than the corresponding given angle. Furthermore it may be seen that when the worms were placed at the given angles 0° and 180° they deviate nearly equally from the given direction. Similar phenomena are observable also at the given angles 45° and 135°. These facts seem to show that when the worms were illuminated from the ventral side of the body they show a kind of random movements.

The changes in the magnitude of angles are shown in Table 49. In the table the positively moved angles show the increasing magnitude while the negatively moved angles show the decreasing magnitude with the increasing magnitude of the given angle, and consequently the average moved angles show that the given angle 90° causes random movements while the smaller given angle causes the stronger negative phototaxis and the larger given angle causes the stronger positive phototaxis.

We may conclude from Table 48 and 49 that even worms unoperated upon, when the brain was less stimulated than the ventral nerve cord, can show random movements from the antagonism between the functioning of the brain and of the ventral nerve cord. We may infer furthermore that when the prostomium was removed and the worms were laterally illuminated they can show random movements at each given angle because the removal of the prostomium causes the feebler functioning of the brain to keep the balance with the functioning of the ventral nerve cord, but when the worms unoperated upon were illuminated ventrally the nervous portions function differentially according to the magnitude of the given angles and consequently the worms show random movements only at the given angle 90°.

It is very interesting to quote Hess's statements (1924) on Lumbricus terrestris. His statements run as follows: " If the ventral nerve cord is severed between the fourth and fifth segments, a rather peculiar condition exists so far as the reactions of these animals to light is concerned. As a rule, these mutilated animals elevate the anterior portion of their bodies as they crawl, often as far caudal as the tenth segment, and when they do so the movements (reactions) are usually

TABLE 49.

	Average		Positive		Negative	
	Occupied angles	Moved angles	Occupied angles	Moved angles	Occupied angles	Moved angles
	in degrees	in degrees	in degrees	in degrees	in degrees	in degrees
0°	30.98	-30.98	0.00	0.00	30.98	-30.98
45°	62.14	-17.14	26.38	8.62	70.76	-25.76
90°	89.96	0.04	74.88	15.12	105.08	-15.08
135°	129.82	5.18	115.88	19.12	148.94	-13.94
180°	148.74	31.26	148.74	31.26	180.00	0.00

positive. If, however, the anterior portion of the body remains in contact with the substratum, the reactions are often negative. This condition is due to the fact that after the operation the first four segments of the animal, that portion anterior to the incision, react negatively to such light while that portion posterior to the incision react positively. ...... Some evidence of the double nature of reactions was observed in those worms described above in which both connectives were severed near the cerebral or the subpharyngeal ganglia. In such worms the prostomium and the first two segments appeared to turn negatively to light, while the portion posterior was definitely positive. Worms in which the ventral nerve cord was severed at the posterior edge of segments 8, 12, 15, 20, and 40, all reacted much the same to lateral illumination of 785 m.c. as when the cord was cut between segments 4 and 5. The general direction of movement for all of these worms was negative since the portion of the animal anterior to the incision was usually sufficient to control the direction in which the animal moved. The posterior portion of earthworm, with the ventral nerve cord cut between segments 20 and 21 or between 40 and 41, reacted much less positively than did those worms in which the cord was cut further anteriorly. As a matter of fact, the ability of the posterior portions of these worms to react (positively) appears to diminish directly as the distance of the incision from the cerebral ganglion of the animal. ...... This leads us to conclude that the cerebral ganglion is the controlling factor in negative reactions."

GENERAL CONCLUSIONS.

1. The orientation of the worms are determined by the antagonistic functioning of the brain and of the ventral nerve cord (Chapters VII, VIII and IX).
2. The brain causes negative orientation and forward crawling, while the ventral nerve cord causes positive orientation crawling either forward or backward (Chapters VI, VII and VIII),
3. By the predominate functioning of the brain, the longer exposure to light of stronger intensity causes the stronger negative phototaxis (Chapters II, III and V), and the same intensity of light causes the strongest negative phototaxis at an elevation angle of about 25° (Chapter IV).
4. The worms show a reaction against the after-effect of removed light (Chapter V).
5. A longer ventral nerve cord causes a stronger positive orientation of the worms. The anteriormost portion of the ventral nerve cord causes the strongest positive orientation, and more posteriorly the positive orientation becomes less pronounced (Chapter VII).

Here I beg to express my best thanks to Prof. Hatai for helpful advice and for kindly giving me access to his library. 1925.

LITERATURE CITED.

1) ADAMS, G. P. 1903 On the negative and positive phototropism of the earthworm *Allolobophora foetida* (SAV.) as determined by light of different intensities. Amer. Jour. Physiol., Vol, 9, pp. 26–34.
2) BARROWS, W. E. 1912 Light, photometry and illumination. New York.
3) HARPER, E. H. 1905 Reactions to light and mechanical stimuli in the earthworm, *Perichaeta bermudensis* (BEDDARD). Biol. Bull., Vol. 10, pp. 17–34.
4) HERMS, W. B. 1911 The photic reactions of sarcophagid flies, especially *Lucilia caesar* LINN. and *Calliphora vomitoria* LINN. Jour. Exp. Zool., Vol. 10, pp. 167–226.
5) HESS, W. N. 1924 Reactions to light in the earthworm, *Lumbricus terrestris* L. Jour. Morph. Physiol., Vol. 39, pp. 515–542.
6) HOLMES, S. J. 1905 The selection of random movements as a factor in phototaxis. Jour. Comp. Neurol. Psychol., Vol. 15, pp. 98–112.

7) JENNINGS, H. S. 1906 Modifiability in behavior. II. Factors determining direction and character of movements in the earthworm. Jour. Exp. Zool., Vol. 3, pp. 435-455.

8) MAST, S. O. 1911 Light and the behavior of organism. New York.

9) SMITH, A. C. 1902 The influence of temperature, odors, light and contact on the movements of the earthworm, *Allolobophora foetida*. Amer. Jour. Physiol., Vol. 6, pp. 460-486.

10) PARKER, G. H. and ARKIN, L. 1901 The directive influence of light on the earthworm *Allolobophora foetida* (SAV). Amer. Jour. Physiol., Vol. 4, pp. 151-157.

11) PARKER, G. H. and PARSHLEY, H, M. 1911 The reaction of earthworms to dry and to moist surfaces. Jour. Exp. Zool., Vol. 11, pp. 361-363.

東北帝國大學

理科報告、

(生　物　學)

第二巻

THE

SCIENCE REPORTS

OF THE

TÔHOKU IMPERIAL UNIVERSITY

FOURTH SERIES

(Biology)

SENDAI, JAPAN

Vol. II.

On Sale by
MARUZEN COMPANY, LTD., TOKYO AND SENDAI.

1927

CONTENTS

PAGE

Effect of Chemicals on Phototaxis in Allolobophora foetida (Sav.), and its Analysis Based upon the Antagonistic Relation between the Functionings of the Brain and of the Ventral Nerve Cord.
By Ekitaro Nomura. 1

The Influence of Coastal Waves on the Shell-Development in Littorina sitchana Phil.
By Ekitaro Nomura. 53

An Application of $a=kb^x$ in Expressing the Growth Relation in the Freshwater Bivalve, Sphaerium heterodon Pils.
By Ekitaro Nomura. 57

Further Studies on the Applicability of $a=kb^x$ in Expressing the Growth Relations in Molluscan Shells. With Four Plates.
By Ekitaro Nomura. 63

Some Preliminary Notes on the Behavior of the Holothurian, Caudina chilensis (J. Müller).
By Toshihiko Yamanouchi. 85

Embryologische Studien an Heloniopsis breviscapa.
Von Tomowo Ono. 93

Effect of Heat on Phototaxis in Allolobophora foetida (Sav.), and its Analysis Based upon the Antagonistic Relation between the Functionings of the Brain and of the Ventral Nerve Cord.
By Ekitaro Nomura and Shinryo Ohfuchi. 105

On the Fertilizin of the Oyster, Ostrea circumpicta.
By Arata Terao. 127

The Influence of Oxygen Tension on the Rate of Oxygen Consumption in Caudina.
By Shichiroku Nomura.. 133

Über die Leibeshöhlenflüssigkeit von Holothurien, Caudina chilensis (J. (Müller).
Von Katsumi Okazaki und Tatsuo Koizumi. 139

Some Preliminary Studies of the Influence upon Plants of the Relative Length of Day and Night. With Four Plates.
By Yoshiji Yoshii.. 143

Grossenverhältnis der Geschlechtschromosomen von Rumex Acetosa, L.
Von Tomowo Ono. 159

Yeasts Isolated from Flower Nectar. With Six Tables and Two Plates.
By Tadao Jimbo. 161

PAGE

Triploidy of Chromosomes in Garden Varieties of Primula Sieboldii, E. Morr.
By Masajiro Iinuma 189

On the Growth Relation in Earshells. With One Plate.
By Kiichiro Sasaki 197

Report of the Biological Survey of Mutsu Bay.
1. General Outline of the Biological Survey of Mutsu Bay.
By Sanji Hozawa 209

Report of the Biological Survey of Mutsu Bay.
2. Medusae of Mutsu Bay.
By Tohru Uchida 215

The Anatomy of Caudina chilensis (J. Müller) with Especial Reference to the Perivisceral Cavity, the Blood and the Water Vascular Systems in their Relation to the Blood Circulation. With Three Plates.
By Nobuyuki Kawamoto 239

Physiological Characteristics of Caudina Muscle, with some Accounts on the Innervation.
By Lieh Tao 265

Bastardierung als eine Ursache für die Entstehung der Chromosomenpolyploidie.
I. Bastard zwischen Chrysanthemum marginatum und C. lavandulaefolium.
Von Masato Tahara u. Naomasa Shimotomai 293

Physiological Studies on the Rhythmical Heart Pulsation in the Oyster, Ostraea circumpicta Pils. With Two Plates.
By Shunichi Takatsuki 301

Contribution to the Research on the Respiration of Fishes.
1. On the Hydrogen Ion Concentration and the CO_2-gas Content and Capacity of Fish Blood.
By Seiji Kokubo 325

On Two New Species of Mantispidae from Japan.
By Masayasu Yazaki 361

Sur la Croissance Normale du Paramecium caudatum.
By Fukuichiro Mizuno 367

Report of the Biological Survey of Mutsu Bay.
3. Notes on the Protozoan Fauna of Mutsu Bay. I. Peridiniales.
By Tohru Hidemiti Abé 383-438

Effect of Chemicals on Phototaxis in *Allolobophora foetida* (SAV.), and its Analysis Based upon the Antagonistic Relation between the Functionings of the Brain and of the Ventral Nerve Cord.

BY

EKITARO NOMURA.

1893 LOEB found that positive phototaxis in *Polygordius* larvae and negative phototaxis in marine copepods could be reversed by means of changing the concentration of sea-water.

1904 LOEB also found that negative phototaxis in freshwater crustaceans, *Gammarus*, *Cyclops*, and *Daphnia*, was reversed by chemicals. "I pointed out," writes LOEB, "that in all probability the light produced chemical changes in the eye or skin of the animals, and that these changes were responsible for the heliotropic reactions. It seems to me that if this were true it should be possible to control the heliotropic phenomena very easily by chemical means.......The fact that such substances as are produced in the animal body itself, e.g., CO_2 or other acids or ammonia, influence the reactions of the animals to light, may explain a number of peculiarities in the heliotropic behavior which we have thus far not been able to understand. The fact that during the daytime the algae at the surface of lakes consume the CO_2, may contribute toward making these animals negatively heliotropic and thus forcing them to migrate downward."

1912 and 1914 ALLEE studied rheotaxis in isopods. "The rheotactic reaction," writes ALLEE, "is an expression of the metabolic condition (physiological state) of the isopod and may be controlled by these factors known to control animal metabolism."

1915 PHIPPS studied phototaxis in amphipods, *Gammarus faciatus*, *Hyallela knickerbockeri*, and *Eucrangonyx gracilis*. "Anything which disturbs the rate of metabolism of the animal," writes PHIPPS, "alters the response to stimuli."

1915 ALLEN studied rheotaxis in *Planaria dorotocephala*. "Reversal of reaction from positive to negative," writes ALLEN, "can be induced by changing the composition of the water; and from negative to positive by return to the former condition."

1918 KANDA studied geotaxis in *Paramecium*. "So-called reversibility of the negative geotropism of *Paramecium caudatum* by chemicals," writes KANDA, "is extremely doubtful. Mechanical 'shock' or agitation is chiefly responsible for the reversal one sees in such cases."

1919 KANDA studied phototaxis in *Arenicola* larvae. "These chemicals which produce artificial parthenogenesis in sea-urchin and other eggs," writes KANDA, "have also a reversing effect on the heliotropism of *Arenicola* larvae."

The object of the present paper was to note the differential influence of chemicals on photic orientations in the brain and the ventral nerve cord of *Allolobophora foetida* found in Sendai (SASAKI, 1924), and to throw, if possible, some light on the investigation of the nature of reversal phototaxis.

For the purpose of investigation, hydrochloric acid, potassium cyanide, strychnine, strychnine nitrate, and strychnine sulphate were used, and for each chemical two kinds of experiments were carried on, one to test the movements of worms from which the anterior 4–5 segments were removed, and the other to test the movements of worms which were not operated upon.

Each fresh worm was washed in drinking water, after being submerged in a solution of chemicals for a definite duration, and then the anterior 4–5 segments were removed, if this operation were needed. In both the cases of operation and of non-operation, a 1% aqueous solution of methylene blue was applied to the worm, and then the worm was placed on a paper which was previously well moistened and spread over a glass plate, its body being straightene at right angles to the light rays coming from an electric lamp of 1000 watts which was put at a distance of 2 meters and at an elevation angle of 25° from the middle of the glass plate. Methylene blue stamped precisely the track of the worm on the surface of the paper (HERMS, 1911).

The definition of positive and negative phototaxis, of positive and negative orientation, of the average, positive, and negative occupied angle, of the average, positively, and negatively moved angle, as well as of the given angle, were made according to NOMURA (1926). In the present investigation the magnitude of the given angle was invariably 90°. Let the total number of individuals which were tested be t, the number of individuals which showed positive orientation or occupied angles smaller than 90° be p, and the number of individuals which showed negative orientation or occupied angles larger than 90° be n. Then

$$p+n=t,$$

$$\text{Average occupied angle}=\frac{\text{The sum of the angles occupied by } t \text{ individuals}}{t}$$

$$\text{Positive occupied angle}=\frac{\text{The sum of the angles occupied by } p \text{ individuals} + 90^\circ n}{t}$$

$$\text{Negative occupied angle}=\frac{\text{The sum of the angles occupied by } n \text{ individuals} + 90^\circ p}{t}$$

$$\text{Average occupied angle} - \text{Positive occupied angle} + 90^\circ = \text{Negative occupied angle}.$$

The direction or the orientation of the crawl was determined at two points of the track: one at the point of the track intersected by the circumference of a circle of 5 cm. radius and the other at the point of the track intersected by the circumference of a circle of 10 cm. radius, the common center being located at the point of origin of the crawl. The former angle is called for convenience the *5 cm. angle* and the latter the *10 cm. angle*. When the worm crawled forward the point of origin of the crawl was located at the anterior end and when it crawled backward at the posterior end of the body.

The arrangement of subjects is as follows:

Chapter I. The effect of hydrochloric acid.
Chapter II. The effect of potassium cyanide.
Chapter III. The effect of strychnine.
Chapter IV. The effect of strychnine nitrate.
Chapter V. The effect of strychnine sulphate.
Chapter VI. Summary and general conclusions.

Chapter I. The effect of hydrochloric acid.

Freshwater *Cyclops*.

Loeb (1904). "*Cyclops* can be made energetically positively heliotropic by CO_2 or any of the other acids effective in *Gammarus*. Positively heliotropic *Cyclops* are made negatively heliotropic by NaHO."

Daphnia.

LOEB (1904). "I have not yet made many experiments with *Daphnia*, and can thus far only state that they can be rendered very outspokenly positively heliotropic by CO_2 or any other acid or by ethylacetate."

MOORE (1912a). "It has been shown by LOEB that the rays of a HERAEUS mercury arc cause *Balanus* larvae and *Daphnia* to become negatively phototropic. This effect is due principally to the ultra-violet rays given off by the mercury lamp, ... Furthermore, I found that the negative effect of ultra-violet light disappears when acids, especially CO_2, are added in small quantities to the water containing the *Daphnia* while the latter are undergoing exposure to the ultra-violet light. If the negative collection is allowed to form, and then to the 20 cc. of water containing the animals there be added 2 cc. of carbonated water, a complete positive collection of the *Daphnia* at once occurs and remains for ten to thirty minutes, after which they may again become negative. The same effect may be produced by substituting 1 cc. N/10 HCl for the carbonated water....... It is evident, however, that the acids which LOEB found would cause *Daphnia* to become positive to visible light, are effective in making these animals positive to the ultra-violet light."

Cypridina.

KANDA (1919) found that negatively strong phototaxis of *Cypridina* could be made positive by HCl or H_2SO_4 solution.

Nauplii of *Balanus perforatus.*

EWALD (1912) "I found that the mineral acids HCl, H_2SO_4 and HNO_3 had a positivating effect, but that the effective concentration had very narrow limits."

MOORE (1912a) obtained the same results as in *Daphnia.*

Gammarus pulex.

LOEB (1904). "*Gammarus pulex* is naturally negatively heliotropic...... If CO_2 is allowed to bubble through the water, or if the animals are thrown into water previously charged with CO_2, they become at once outspokenly positively heliotropic. Other acids, e. g., HCl, or oxalic or acetic, act in the same way. A concentration of about m/500 is sufficient for this effect."

Hyallela knickerbockeri.

JACKSON (1910) obtained similar results to those of LOEB (1904), on *Gammarus pulex.*

Nymphs of *Heptagenia interpunctata.*

WODSEDALEK (1911). "Of the different classes of chemicals employed the acids were the most effective in reversing the phototaxis of *H. interpunctata* nymphs. A large majority, and in some cases all of the twenty specimens became positive in the acid solutions. When CO_2 was bubbled through the water, again, a large percentage of the specimens became positive. The concentrations sufficient for this effect with the different acids used are, — HCl 0.04%, tartaric 0.04%, HNO_3 0.03%, H_2SO_4 0.03%, boracic 0.05%, and acetic 0.07%."

Arenicola larvae.

KANDA (1919). "In 0.0036 m. HCl solution made with sea-water, about 80% of the larvae became negative and in similar solutions of H_2SO_4 about 95% became negative 15 minutes after the treatment at 21°C."

A 1/100 normal solution of HCl was used in the present investigation on *Allolobophora foetida.*

When the worms were submerged in this solution, they showed convulsion, which continued for 8 or more seconds. In some worms 7 seconds were sufficient to cause convulsion, while in others even 30 seconds were not enough, though in most cases the worms began to convulse at durations of submergence from 11 to 17 seconds.

After an elapse of 3–6 seconds from the beginning of convulsion, most of the worms began to eject hypodermal secretion, but in some cases the ejection occurred previous to, or at the same time with, the beginning of convulsion.

A longer duration of submergence caused a stronger contraction of circular muscle, a stronger relaxation of longitudinal muscle, and more frequent posterior movements of the worms, but all the worms could definitively crawl forward for a requisite distance even when they were submerged in the solution for 40 seconds.

THE MOVEMENTS OF UNOPERATED WORMS.

Eight tests in which worms were submerged individually in a definite solution of HCl for a duration of 0, 5, 10, 15, 20, 25, 30, or 40 seconds were carried on during the middle part of August in the dark room, the temperature of which was 25.5°-26.5°C., and for each test 50 worms were used.

When the worms were submerged for 45 seconds, they showed wriggling or squirming movements and some of them could not perfectly describe their tracks.

The orienting or the phototactic movements of the worms are shown in Tables 1 and 2.

TABLE 1.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
0 sec.	6	9	35	7	5	38
5 sec.	7	11	32	8	7	35
10 sec.	10	9	31	11	6	33
15 sec.	10	10	30	12	6	32
20 sec.	11	9	30	11	9	30
25 sec.	12	10	28	12	11	27
30 sec.	10	11	29	11	7	32
40 sec.	5	7	38	9	6	35

In Table 1 the frequency distribution of the worms is given in absolute numbers in the angular sections 0°-80°, 81°-99°, and 100°-180°. In this table it may be thought in general that the number of positively orienting individuals is given in the section 0°-80°, the number of negatively orienting individuals in the section 100°-180°, and the number of indistinctly or indifferently orienting individuals in the section 81°-99°. The following facts may be seen in the table:

1. In both the 5 cm. and the 10 cm. angles, the increase of the number of seconds from 0 to 25 causes the gradual decrease of the number of negatively orienting individuals, while the increase of the number of seconds above 25 causes the gradual increase. This fact shows that when the worms are submerged in the solution for a longer duration the degree of negative orientation in the worms becomes weaker, but a duration of submergence longer than 25 seconds makes the degree stronger again.

2. In the 5 cm. angles, the number of positively orienting individuals tends to increase with the increase of the number of seconds

from 0 to 25, but it decreases when the number of seconds increases from 25 to 40. In the 10 cm. angles, the increase of the number of seconds from 0 to 10 or 15 causes the gradual increase of the number of positively orienting individuals, while the increase of the number of seconds from 25 to 40 causes the gradual decrease of the number of positively orienting individuals, and the increase of the number of seconds from 10 or 15 to 25 causes no distinct alterations of the number of positively orienting individuals. From these facts we may conclude that when the worms are submerged in the solution for a duration from 0 to 15 or more seconds the degree of positive orientation in the worms becomes stronger with the increase of the number of seconds, while the degree becomes weaker again when the number of seconds increases from 25 to 40.

3. In both the 5 cm. and the 10 cm. angles, the number of negatively orienting individuals is invariably greater than the sum of the numbers of positively and of indifferently orienting individuals, showing that even when the worms are submerged in the solution of HCl for any duration they exhibited negative orientation as a whole.

In Table 2 the changes in the magnitude of the occupied angles are shown and from this table Fig. 1 was plotted in order to see easily the state of the changes. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, the magnitude of the negatively moved angle is invariably greater than that of the corresponding positively moved angle, consequently the average moved angle shows the negativity of the worms which were not operated upon.

2. The increase of the number of seconds from 0 to 25 causes a slower decrease of the magnitude of the average, as well as of the negative, occupied angles, but the increase above 25 causes a quicker increase of the magnitude of these angles. A similar tendency of changes may be seen also in the positive occupied angles, though in this case the magnitude of these angles show only a slight alteration. We may infer that the changes in the average angles are mainly influenced by the changes in the negative angles.

From Tables 1 and 2 we may conclude that when the worms are submerged in the 1/100 normal solution of HCl and when they are not operated upon, their degree of negative orientation becomes weaker

TABLE 2.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 sec.	85.04	116.40	121.36	83.18	124.24	131.06
5 sec.	84.88	112.48	117.60	82.26	122.44	130.18
10 sec.	82.98	109.00	116.02	82.92	121.20	128.28
15 sec.	82.64	109.48	116.84	81.62	116.26	124.64
20 sec.	82.54	108.62	116.08	82.00	116.02	124.02
25 sec.	82.64	107.76	115.12	81.26	114.36	123.10
30 sec.	82.08	114.52	122.44	81.74	123.46	131.72
40 sec.	84.04	128.60	133.56	83.70	131.34	137.64

FIG. 1.

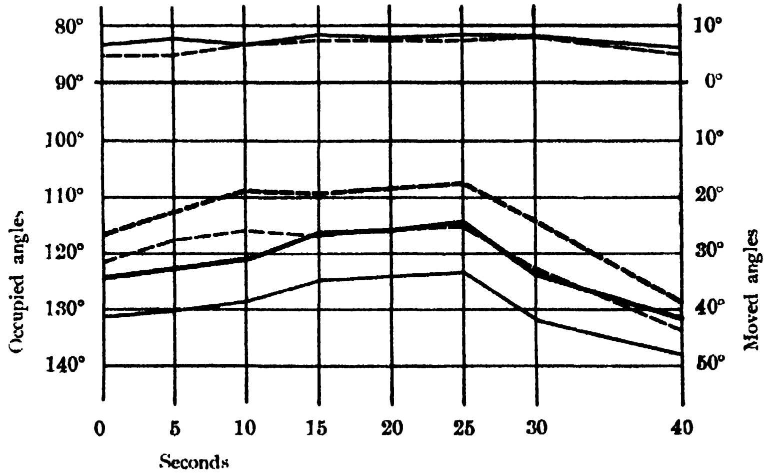


Fig. 1. The given angle 90° or the moved angle 0° is denoted by the horizontal line 90°–0°, and the moved angles upward from this line show the positively and those downward the negatively moved angles. The tracings of the average angles are denoted by the thicker lines, and those of the positive and negative angles by the thinner lines. The tracings of the 5 cm. angles are denoted by the broken lines and those of the 10 cm. angles by the full lines. All the figures in this paper, except 3, 6, 9, 12, and 15, are plotted in the same way.

slowly with the prolongation of the duration of submergence from 0 to about 25 seconds, but when the duration of submergence is prolonged beyond this, it becomes stronger again.

THE MOVEMENTS OF OPERATED WORMS.

Five tests were carried on during the latter part of August at durations of submergence of 0, 10, 20, 30, and 40 seconds, and in each test 25 worms were used. The temperature in the dark room was also 25.5°–26.5°C,

TABLE 3.

Duration of submergence	5 cm. angles		10 cm. angles	
	Forward	Backward	Forward	Backward
0 sec.	9	16	10	15
10 sec.	12	13	11	14
20 sec.	5	20	5	20
30 sec.	10	15	9	16
40 sec.	7	18	7	18
Average	8.6	16.4	8.4	16.6

The absolute numbers of forward and backward crawling individuals are given in Table 3. From this table I am not able to find any regularities in the arrangement of numbers, but I may mention that nearly 1/3 of the worms crawled forward, while nearly 2/3 of them crawled backward, irrespective of the duration of submergence.

The absolute numbers of positively, indifferently, and negatively phototactic individuals are given together in Table 4. In this table the forward crawling individuals which made the occupied angles from 0° to 80°, as well as backward crawling individuals which made the occupied angles from 180° to 100°, are taken as the positively phototactic individuals; the forward crawling individuals which made the occupied angles from 100° to 180°, as well as the backward crawling individuals which made the occupied angles from 80° to 0° as the negatively phototactic individuals; and the remaining, forward or backward crawling individuals as the indifferently phototactic individuals. From this table, also, I am not able to find any regular arrangements of the numbers of individuals, but I may mention that the number of individuals which crawled backward toward the light (positively phototactic, but negatively orienting) tended to increase according to the prolongation of the duration of submergence, while the number of individuals which crawled backward away from the light (negatively

TABLE 4.

	Duration of submergence	Positive Phototaxis			Indifferent Phototaxis			Negative Phototaxis		
		Forward crawling 0°–80°	Backward crawling 180°–100°	Sum	Forward crawling 81°–99°	Backward crawling 99°–81°	Sum	Forward crawling 100°–180°	Backward crawling 80°–0°	Sum
5 cm. angles	0 sec.	7	1	8	1	1	2	1	14	15
	10 sec.	11	2	13	0	1	1	1	10	11
	20 sec.	4	4	8	0	3	3	1	13	14
	30 sec.	8	4	12	0	3	3	2	8	10
	40 sec.	3	6	9	0	3	3	4	9	13
	Average			10.0			2.4			12.6
10 cm. angles	0 sec.	7	0	7	1	1	2	2	14	16
	10 sec.	11	2	13	0	1	1	0	11	11
	20 sec.	4	3	7	1	4	5	0	13	13
	30 sec.	9	4	13	0	4	4	0	8	8
	40 sec.	3	10	13	0	1	1	4	7	11
	Average			10.6			2.6			11.8

phototactic, but positively orienting) tended to decrease, and that the degree of negative phototaxis in the worms which were operated upon was pronounced nearly equally to, or slightly stronger than, the degree of positive phototaxis.

The orienting movements of the worms which were operated upon are shown in Tables 5 and 6.

TABLE 5.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0 sec.	21	2	2	21	2	2
10 sec.	20	2	3	20	3	2
20 sec.	17	5	3	17	5	3
30 sec.	17	3	4	17	4	4
40 sec.	13	2	10	10	1	14

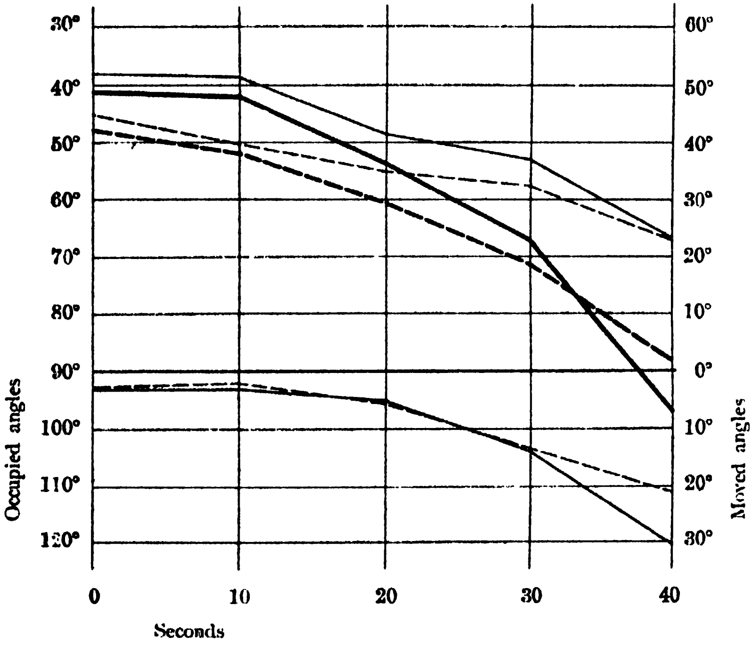
In Table 5 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in this table:

1. In both the 5 cm. and the 10 cm. angles, the increase of the number of seconds causes the decrease of the number of positively orienting individuals (section 0°–80°) and the increase of the number

TABLE 6.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 sec.	44.94	47.72	92.78	38.28	41.20	92.92
10 sec.	50.28	52.16	91.88	39.20	42.24	93.04
20 sec.	55.04	60.56	95.52	48.68	53.76	95.08
30 sec.	57.44	71.16	103.72	52.80	66.84	104.04
40 sec.	66.84	88.64	111.80	66.64	96.92	120.28

FIG. 2.



of negatively orienting individuals (section 100°-180°).

2. In both the 5 cm. and 10 cm. angles, the number of positively orienting individuals is greater than that of negatively orienting individuals at durations of submergence of 0, 10, 20, and 30 seconds, but at 40 seconds the number of negatively orienting individuals becomes close to, or greater than, that of positively orienting individuals.

In Table 6 the changes in the magnitude of the occupied angles-are shown and from this table Fig. 2 was plotted. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, at durations of submergence shorter than 30 seconds, the magnitude of the positively moved angle is larger than that of the corresponding negatively moved angle, consequently the average moved angle shows the positivity of the worms which were operated upon, but at the submergence of 40 seconds the difference between the positively and the negatively moved angles becomes small, consequently the average moved angle shows that the orientation of the worms is obscure or indeterminable in this case.

2. In both the 5 cm. and the 10 cm. angles, the magnitude of the positively moved angle or the positivity of the worms decreases with the prolongation of the duration of submergence, while the magnitude of the negatively moved angle or the negativity of the worms increases. In consequence, the magnitude of the average moved angle shows an accelerative decrease of the positivity of the worms, till finally, at the duration of submergence of 40 seconds, the orientation of the worms becomes obscure or negative.

From Tables 5 and 6 we may conclude that when the worms are submerged in the 1/100 normal solution of HCl and when they are operated upon, their degree of positive orientation becomes weaker with the prolongation of the duration of submergence, and, in turn, their degree of negative orientation becomes stronger. Moreover, from this relation and from Table 4, we may infer that the solution of HCl weakens the positively orienting functioning of the ventral nerve cord, while it does not influence so much the negatively orienting functioning, consequently, in the ventral nerve cord the negativity increases according to the prolongation of the duration of submergence, while the positivity decreases.

THE CHANGES OF NEGATIVITY IN THE BRAIN.

In my previous paper (NOMURA, 1926) it was concluded that the photic orientation of *Allolobophora foetida* was determined by the antagonism between the functionings of the brain and of the ventral nerve cord.

Now let the degree of photic orientation of the worms be A, the degree of negative orientation which is exhibited by the functioning of the brain be N, and the degree of positive orientation which is exhibited by the functioning of the ventral nerve cord be P. Then from the antagonistic relation we find the equation

$$A - P + 90^\circ = N,$$

FIG. 3.

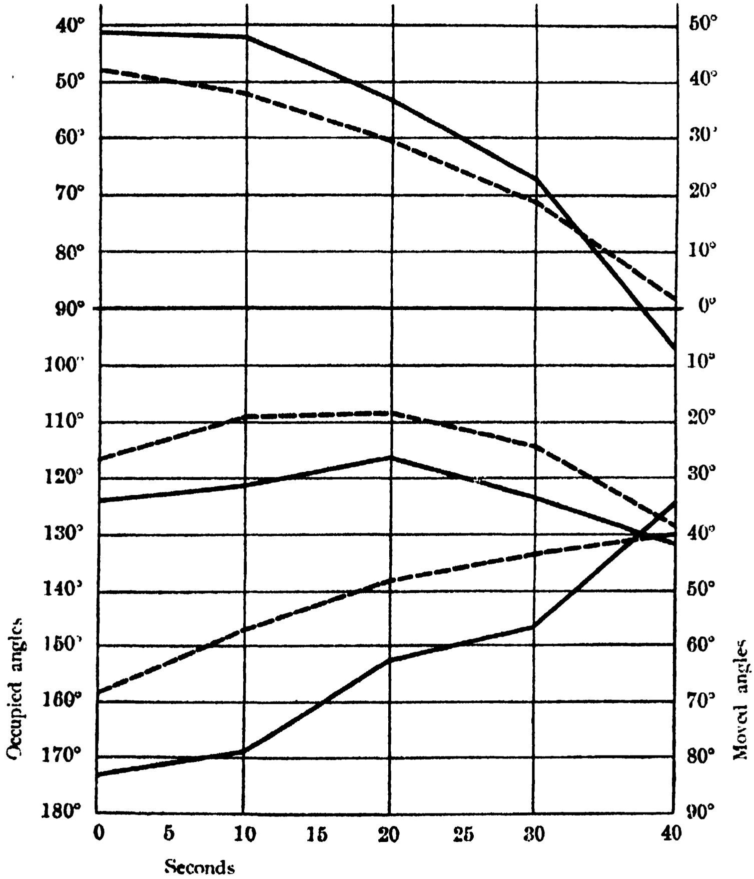


Fig. 3. The tracings of the 5 cm. angles are denoted by the broken lines and those of the 10 cm. angles by the full lines. Figs. 6, 9, 12, and 15 are plotted in the same way.

TABLE 7.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	P	*A*	*N*	*P*	*A*	*N*
0 sec.	47.72	116.40	158.68	41.20	124.24	173.04
10 sec.	52.16	109.00	146.84	42.24	121.20	168.96
20 sec.	60.56	108.62	138.06	53.76	116.02	152.26
30 sec.	71.16	114.52	133.36	66.84	123.46	146.62
40 sec.	88.64	128.60	129.96	96.92	131.34	124.42

in which 90° is the magnitude of the given angle in the present experiments.

The values of *A* are directly the magnitudes of the average occupied angles made by the worms which were not operated upon, and the values of *P* may be assumed in probable approximation to be the magnitudes of the average occupied angles made by the worms which were operated upon. Then from the data given for the operated and the unoperated worms, we may calculate the probable values of *N*.

In Table 7 the values of *P*, *A* and *N* are given together, and from this table Fig. 3 was plotted in order to see easily the state of the changes. It may be seen in the figure that when the worms are submerged in the 1/100 normal solution of HCl, the degree of negative orientation which is exhibited by the brain becomes weaker with the prolongation of the duration of submergence.

It is worthy of note that the degree of negative orientation in the unoperated worms becomes more pronounced with the prolongation of the duration of submergence from about 25 to 40 or more seconds, because the degree of negative orientation becomes more pronounced (Tables 4, 5, and 6) in the ventral nerve cord, in spite of the degree of negative orientation becoming less pronounced in the brain.

CONCLUSIONS.

1. In the ventral nerve cord, HCl causes an accelerative weakening of the positively orienting functioning and, in turn, a strengthening of the negatively orienting functioning.

2. In the brain, HCl causes a weakening of the negatively orienting functioning and a weakened inhibition of the elongation of the posterior end of the body.

3. In consequence, in worms HCl causes a retardative weakening at first and then an accelerative strengthening of the degree of negative orientation or phototaxis, and a more frequent elongation of the posterior end of the body.

Chapter II. The effect of potassium cyanide.

1904 Loeb studied phototaxis in *Gammarus pulex.* " Pure oxygen or hydrogen," states Loeb, " does not make them positively heliotropic, but leaves them markedly negatively heliotropic."

1912 Ewald studied phototaxis in the nauplii of *Balanus perforatus.* " Lack of oxygen," writes Ewald " has a very strong effect on phototaxis. If nauplii are put in sea-water which has been evacuated for a few minutes by means of a filter pump (at about 300 mm. of mercury pressure) they become positive immediately and remain so as long as they are left in the water. Transferred into fresh water they became negative again with equal velocity. This is one of the strongest and most striking reaction. ...... Experiments with potassium cyanide, strychnine, oxygen and hydrogen peroxide met with no success." According to this statement, lack of oxygan did not show the same results as potassium cyanide.

1912 Allee studied rheotaxis in isopods. " Potassium cyanide," writes Allee, " is known to decrease the amount of oxidation by decreasing the ability of the tissue to take up oxygen. ...... Low oxygen (Haldane and Smith) potassium cyanide (Geppert) and high carbon dioxide (Cushny) do this (depression of the rate of animal metabolism) by decreasing the oxidations. ...... The general conclusion to be drawn from this series of experiments is that in the isopod, *Asellus communis,* the rheotactic reaction is dependent upon the metobolic state of the animal for its degree of positiveness and that the natural or experimental conditions which affect the metabolic state of the animal, change its rheotactic response."

1915 Phipps certificated Allee's view in the study of *Gammarus Hyallela,* and *Eucrangonyx.* " In these experiments on amphipods then, " writes Phipps, " the reversal reactions are caused by some change in the metabolic processes of the animals. KCN depresses the metabolic processes by decreasing oxidation. Oxidation is decreased by decreasing the ability of the tissues to take up oxygen. . . . . . . Such reagents and treatment are known to have specific effects on metabolism and they also cause reversals in phototaxis, therefore the responses are related to the metabolic rate of the animal."

1919 KANDA studied phototaxis in *Arenicola* larvae. "LILLIE oberved," writes KANDA, "one curious effect in larvae treated with one fourth and one fifth saturated solution of chloroform in sea-water; when the larvae, after about two hours in corked flasks containing these solutions, were poured out into watch glasses the great majority were found, after three or four hours (when the chloroform had evaporated), to exhibit a pronounced negative phototaxis in place of the usual positive. . . . . . . As was pointed out in his previous paper, the writer found a similar phenomenon in the larvae treated with sodium, potassium, calcium and magnesium chloride solutions when they were returned into normal sea-water. This was particularly remarkable in the larvae treated with the last two solutions during the actual immersion in which there was never any reversal of positive heliotropism. . . . . . . In the present experiments, the same phenomenon was also found in larvae treated with salts, narcotics—chloroform, chloral and formalin in particular—strong alkalies, potassium cyanide, etc. As already shown, most of these chemicals were not favorable agents for the reversal of the positive heliotropism of the larvae. Moreover, in some cases, KCN, for instance, the larvae remained positive or indifferent to light while in presence of the chemical substance (dissolved in normal sea-water). Nevertheless the 'after-effect' was pronounced. That is to say, almost all larvae became negative when returned to normal sea-water. Such facts are consistent with the hypothesis that these chemicals act by changing in some way the normal properties of the plasma membranes of the irritable elements concerned, so that a certain time is required to recover normal conditions after return to normal sea-water."

1924 BODINE studied physiological actions of cyanides. "The physiological actions of HCN and its salts," writes BODINE, "appear to be due (a) to the ease with which HCN molecules penetrate living cells and their ionizing, exert their influence by means of H ions and CN ions; (b) to the weakness of HCN as an acid, which permits at neutrality or at even slight alkalinity the presence of a considerable amount of free HCN molecules in the presence of their salts; (c) to specific effects occasioned by its chemical activity."

In my present experiments a 1/7 normal solution of KCN was used.

When the worms were submerged into this solution, they showed convulsion which continued for 10 or more seconds. In some worms 5 seconds were sufficient to cause convulsion, while in others even 35 seconds were not enough, though in most cases the worms began to convulse at durations of submergence from 18 to 25 seconds.

The ejection of hypodermal secretion, which is insoluble in the solution and gives a peculiar fetid odor, occurred previous to, at the same time with, or after, the beginning of convulsion.

A longer duration of submergence caused a stronger contraction of circular muscle, a stronger relaxation of longitudinal muscle, a more frequent posterior elongation which often took turns with backward crawling, and an increased amount of slimy hypodermal secretion, which stains deeply with methylene blue.

THE MOVEMENTS OF UNOPERATED WORMS.

Five tests were carried on during the latter part of August in the dark room, the temperature of which was 25.5°–26.5°C. and at each test 50 worms were used after being submerged individually in a definite solution of KCN for a duration of 0, 10, 20, 30, or 35 seconds.

At durations of submergence of 0, 10, 20, and 30 seconds all the worms definitively crawled forward. At a duration of submergence of 35 seconds, when they were tested at the 5 cm. angles, 36 individuals crawled forward while 14 crawled backward, and when they were tested at the 10 cm. angles, 38 individuals crawled forward while 12 crawled backward. But at a duration of submergence of 40 seconds, nearly all the worms could crawl neither forward nor backward owing to the irresponsibility which occurred in the longer posterior portion of the body.

The orienting movements of the worms are shown in Tables 8 and 9.

In Table 8 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in this table:

1. In both the 5 cm. and the 10 cm. angles, a prolongation of the duration of submergence causes a decrease of the number of negatively orienting individuals (section 100°–180°) and an increase of the number of positively orienting individuals (section 0°–80°).

TABLE 8.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0 sec.	6	9	35	7	5	38
10 sec.	6	9	35	11	4	35
20 sec.	11	8	31	11	5	34
30 sec.	17	9	24	17	6	27
35 sec.	22	9	19	20	8	22

2. In both the 5 cm. and the 10 cm. angles, the number of negatively orienting individuals is greater than that of positively orienting individuals at durations of submergence from 0 to 30 seconds, but at 35 seconds the number of negatively orienting individuals becomes close to that of positively orienting individuals.

In Table 9 the changes in the magnitude of the occupied angles are shown and from this table Fig. 4 was plotted. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, at a duration of submergence shorter than 30 seconds, the magnitude of the negatively moved angle is invariably larger than that of the corresponding positively moved angle, consequently the average moved angle shows the negativity of the worms which were not operated upon, but at a duration of submergence longer than 30 seconds the difference between the negatively and the positively moved angles becomes smaller, consequently the average moved angle shows an indeterminable, or a slightly negative, orientation.

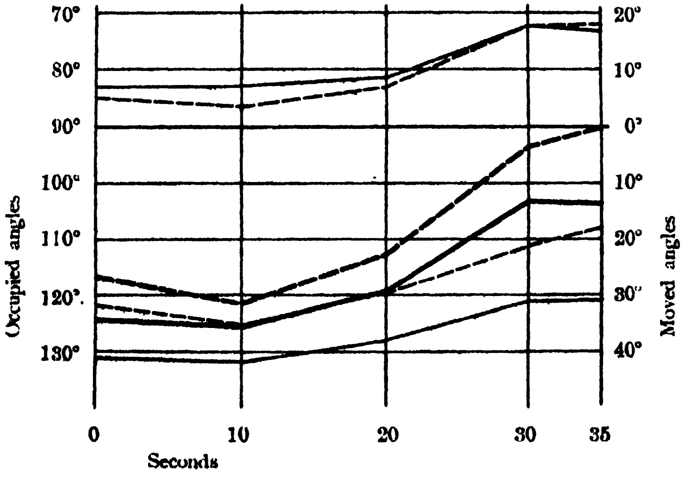
2. In both the 5 cm. and the 10 cm. angles, following the prolongation of the duration of submergence from 0 to 30 seconds, the magnitude of the positively moved angle or the positivity of the worms tends to increase acceleratively, and the magnitude of the negatively moved angle or the negativity of the worms tends to decrease also acceleratively, consequently, the magnitude of the average moved angle

shows an accelerative decrease of the negativity of the worms, but following the prolongation of the duration of submergence above 30 seconds the magnitude of the positively moved angle increases retardatively and that of the negatively moved angle decreases also retardatively, consequently the magnitude of the average moved angle shows a retardative decrease of the negativity of the worms.

TABLE 9.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 sec.	85.04	116.40	121.36	83.18	124.24	131.06
10 sec.	86.50	121.56	125.06	83.34	125.30	131.96
20 sec.	82.98	112.70	119.72	81.66	119.64	127.98
30 sec.	72.72	93.72	111.00	72.60	103.64	121.04
35 sec.	71.98	90.10	108.12	73.52	104.26	120.74

FIG. 4.



From Tables 8 and 9 we may conclude that when the worms are submerged in a 1/7 normal solution of KCN and when they are not operated upon, their degree of positive orientation becomes strengthened while their degree of negative orientation becomes weakened, and the changes in the degrees take place acceleratively when the submergence is prolonged from 0 to about 30 seconds, but retardatively when it is prolonged more.

THE MOVEMENTS OF OPERATED WORMS.

Four tests were carried on during the latter part of August at a duration of submergence of 0, 10, 20 or 30 seconds, and in each test 25 worms were used individually. The temperature of the dark room was also 25.5°-26.5°C.

When the worms were operated upon after being submerged for 35 seconds, many of them could not describe their tracks perfectly.

The absolute numbers of forward and backward crawling individuals are given in Table 10. Though the alterations of the numbers are

TABLE 10.

Duration of submergence	5 cm. angles		10 cm. angles	
	Forward	Backward	Forward	Backward
0 sec.	9	16	10	15
10 sec.	9	16	9	16
20 sec.	9	16	9	16
30 sec.	4	21	4	21

TABLE 11.

	Duration of submergence	Positive Phototaxis			Indifferent Phototaxis			Negative Phototaxis		
		Forward crawling 0°-80°	Backward crawling 180°-100°	Sum	Forward crawling 81°-99°	Backward crawling 99°-81°	Sum	Forward crawling 100°-180°	Backward crawling 80°-0°	Sum
5 cm. angles	0 sec.	7	1	8	1	1	2	1	14	15
	10 sec.	5	3	8	3	4	7	1	9	10
	20 sec.	3	4	7	2	6	8	4	6	10
	30 sec.	1	6	7	2	7	9	1	8	9
	Average			7.5			6.5			11.0
10 cm. angles	0 sec.	7	0	7	1	1	2	2	14	16
	10 sec.	6	5	11	1	2	3	2	9	11
	20 sec.	4	3	7	1	7	8	4	6	10
	80 sec.	3	10	13	1	4	5	0	7	7
	Average			9.5			4.5			11.0

very slight, this table shows the tendency that the frequency of forward crawling decreases according to the prolongation of the duration of submergence, while that of backward crawling increases.

The absolute numbers of positively, indifferently, and negatively phototactic individuals are shown together in Table 11 in the same manner as in Table 4. The following facts may be seen in the table:

1. When the worms are not submerged in the solution the number of negatively phototaxic individuals is greater than the sum of the numbers of positively and of indifferently phototactic individuals, but when they are submerged the reverse takes place, and the difference between the numbers tends to increase with the prolongation of the duration of submergence.

2. The number of individuals which crawl forward toward the light (positively phototactic and positively orienting) tends to decrease with the prolongation of the duration of submergence.

3. The number of individuals which crawl backward toward the light (positively phototactic, but negatively orienting) tends to increase with the prolongation of the duration of submergence, while the number of individuals which crawl backward away from the light (negatively phototactic, but positively orienting) tends to decrease.

From Tables 10 and 11 we may conclude that at any duration of submergence the number of backward crawling individuals is greater than that of forward crawling individuals, and that following the prolongation of the duration of submergence the number of forward crawling individuals, as well as of positively phototactic individuals which crawl forward, tends to decrease, while the number of indifferently and of negatively phototactic individuals which crawl forward tends to increase; but the number of backward crawling individuals, as well as of positively and indifferently phototactic individuals which crawl backward, tends to increase, while the number of negatively phototactic individuals which crawl backward tends to decrease; consequently the number of positively and of indifferently phototactic individuals becomes larger than that of negatively phototactic individuals.

The orienting movements of the worms which were operated upon are shown in Tables 12 and 13.

In Table 12 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

1. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals (section 0°–80°) decreases with the prolongation

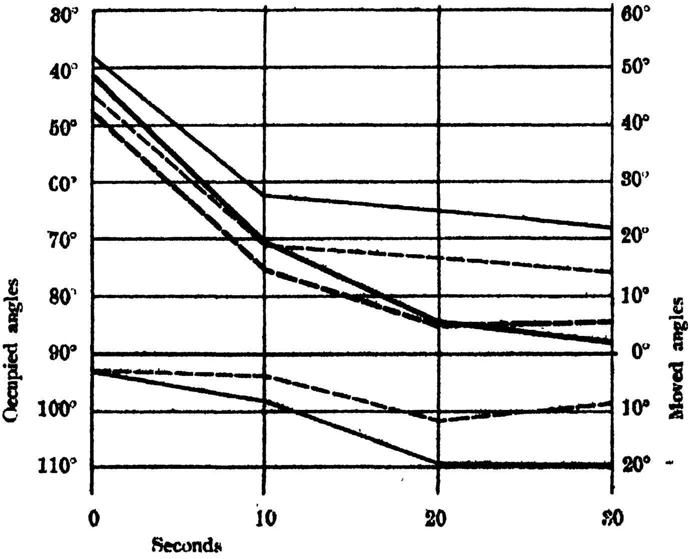
TABLE 12.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0 sec.	21	2	2	21	2	2
10 sec.	15	7	3	15	3	7
20 sec.	9	8	8	10	8	7
30 sec.	9	6	10	10	5	10

TABLE 13.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 sec.	44.94	47.72	92.78	38.28	41.20	92.92
10 sec.	71.20	75.20	94.00	62.32	70.84	98.52
20 sec.	78.32	85.16	101.84	65.20	84.68	109.48
30 sec.	75.72	84.68	98.96	68.32	87.96	109.64

FIG. 5.



of the duration of submergence, while that of negatively orienting individuals (section 100°–180°) increases.

2. In both the 5 cm. and the 10 cm. angles, at the durations of submergence of 0 and 10 seconds the number of positively orienting individuals is greater than that of negatively orienting individuals, but at 20 and 30 seconds the number of negatively orienting individuals becomes close to that of positively orienting individuals.

In Table 13 the changes in the magnitude of the occupied angles are shown and from this table Fig. 5 was plotted. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, at the durations of submergence of 0 and 10 seconds the magnitude of the positively moved angle is larger than that of the corresponding negatively moved angle, consequently the average moved angle shows the positivity of the worms which were operated upon, but at the durations of submergence longer than 30 seconds the difference between the positively and the negatively moved angles becomes small, consequently the average moved angle shows that the orientation of the worms is slightly positive or indeterminable in these cases.

2. In both the 5 cm. and the 10 cm. angles, the magnitude of the positively moved angle decreases retardatively with the prolongation of the duration of submergence, while the magnitude of the negatively moved angle increases somewhat retardatively. In consequence, the magnitude of the average moved angle shows a retardative decrease of the positivity of the worms.

From Tables 12 and 13 we may conclude that when the worms are submerged in the 1/7 normal solution of KCN and when they are operated upon, their degree of positive orientation becomes weaker retardatively in relation to the prolongation of the duration of submergence, and, in turn, their degree of negative orientation becomes stronger.

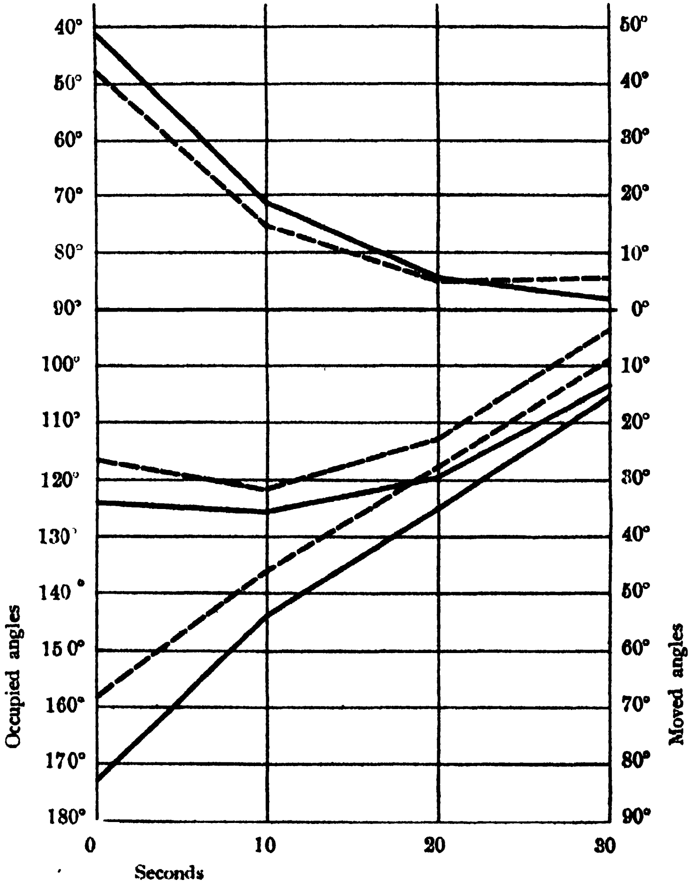
THE CHANGES OF NEGATIVITY IN THE BRAIN.

The magnitudes of the average occupied angle *A* which were made by the unoperated worms, the magnitudes of the average occupied angle *P* which were made by the operated worms, and the calculated magnitudes of *N* which show the degrees of negative

TABLE 14.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	P	*A*	*N*	*P*	*A*	*N*
0 sec.	47.72	116.40	158.68	41.20	124.24	173.04
10 sec.	75.20	121.56	136.36	70.84	125.30	144.46
20 sec.	85.16	112.70	117.54	84.68	119.64	124.96
30 sec	84.68	93.72	99.04	87.96	103.64	105.68

FIG. 6.



orientation in the brain are given together in Table 14, and from this table Fig. 6 was plotted in order to see easily the state of the changes.

In this figure it may be seen that when the worms are submerged in the 1/7 normal solution of KCN the degree of negative orientation which is exhibited by the brain becomes weaker somewhat retardatively according to the prolongation of the duration of submergence.

CONCLUSIONS.

1. In the ventral nerve cord, KCN causes an inhibition of forward crawling, a retardative weakening of the positively orienting functioning, and, in turn, a retardative strengthening of the negatively orienting functioning, till finally the degrees of the functionings balance each other.
2. In the brain, KCN causes a retardative weakening of the negatively orienting functioning and a weakened inhibition of backward crawling.
3. In consequence, in worms KCN causes an accelerative weakening of the degree of negative orientation or phototaxis, till finally the orientation or phototaxis becoms obscure, and a more frequent posterior elongation which often takes turns with backward crawling.

Chapter III. The effect of strychnine.

EWALD (1912) could not reverse negative phototaxis in the nauplii of *Balanus perforatus* to positive by means of strychnine.

MOORE (1912b). "Heretofore, it has not been possible to cause the formation of negative collections of these small crustacea by chemical means. Since the agents which cause such animals to form positive collections are acids and narcotics such as alcohol, ether and esters, it occurred to me that if a substance which has the power of intensifying sensation were applied to the animals we should get as a result a negative collection. It is known that in vertebrates, strychnine has the effect of thus intensifying sensation, especially with reference to light. Accordingly a few drops of a saturated solution of strychnine were added to a dish containing *Daphnia pulex* in normally positive collection. The collection at once broke up and the animals executed rapid circular movements for some hours, but did not tend to collect in any part of the dish. ...... The effect of strychnine was next tried in the same way on *Diaptomus bakeri* (?). In one minute after the addition of the strychnine the animals, normally positive or indifferent, formed a marked negative collection. This collection remained for an hours or longer, depending on the initial condition of the animals. In fact the experiment was uniformly successful only if the *Diaptomus* were used the same day they were taken from the lake. . . . . . . Other excitants for the control nervous system were tried in the same manner. Caffeine caused a positive collection; atropine brought about a marked tendency to form a negative collection but in a much less degree than strychnine. It may be of significance that in vertebrates caffeine acts as an excitant only for the higher centers and strychnine for the lower, while atropine occupies a position midway between the two (CUSHNY, Pharmacology p. 282). . . . . . . The negative phototropism brought about by strychnine may be reversed by the agents which ordinarily cause a positive formation. In addition to acids, camphor, when sprinkled on the surface of the water in the form of gum, reverse the negative phototropism due to strychnine. It is significant that, in the vertebrates, camphor paralyses the central nervous system and in the frog is an antidote for strychnine (CUSHNY, p. 431). While negative phototropism in *Diaptomus* can be reversed by acids and camphor, positive phototropism brought about by chemical means cannot be reversed by strychnine."

CROZIER and FEDERIGHI (1924). "By injection with strychnine the phototropic circus movements of the slug, *Limax maximus*, may be suppressed, its phototropism abolished. The creeping activity of the foot is not in any essential may interferred with. Strychnine produces in *Limax* central nervous effects of the sort associated with its characteristic action. Hence, although an effect of the drug upon photoreceptors cannot be definitely excluded, the experimental result is held to demonstrate that in orientation during circus movements there occurs central 'competition' between impulses resulting (1) in the release of pedal waves and (2) in the maintenance of a turning posture."

In my experiments on *Allolobophora foetida* a saturated solution of strychnine was used. Strychnine is only slightly soluble, requiring 7000 parts of water for solution.

Even when the worms were submerged in this solution for a long duration, nearly all the worms showed neither convulsion nor ejection of hypodermal secretion.

THE MOVEMENTS OF UNOPERATED WORMS.

Eight tests were carried on during October 17 to 21 in the dark room, the temperature of which was 17.0°-17.8°C., and at each test 50 worms were used after being submerged individually in the definite solution of strychnine for a duration of 0, 3, 6, 10, 15, 20, 25, or 30 minutes.

At any duration of submergence all the worms crawled forward.

The orienting movements of the worms are shown in Tables 15 and 16.

In Table 15 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

TABLE 15.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
0 min.	11	12	27	7	13	30
3 min.	12	13	25	8	10	32
6 min.	18	12	20	13	5	32
10 min.	21	12	17	15	14	21
15 min.	24	13	13	18	12	20
20 min.	24	15	11	24	9	17
25 min.	26	13	11	25	12	13
30 min.	26	11	13	19	12	19

1. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals (section 0°-80°) tends to increase with the prolongation of the duration of submergence from 0 to 25 minutes, while that of negatively orienting individuals (section 100°-180°) tends to decrease; but further prolongation of the duration tends to cause a decrease of the number of positively orienting individuals and an increase of negatively orienting individuals.

2. In the 5 cm. angles, at the durations of submergence of 0 and 3 minutes, the majority of worms show negative orientation, but at durations from 10 to 25 minutes they come to show positive orientation, though at a duration of 30 minutes the number of negatively orienting

individuals tends to increase.

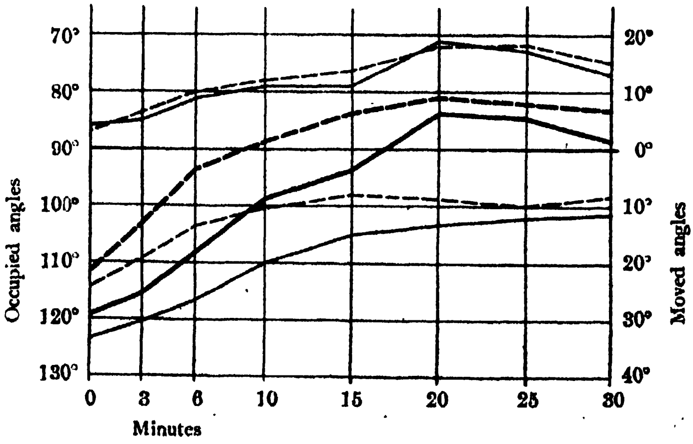
3. In the 10 cm. angles, at durations of submergence from 0 to 10 minutes, the majority of worms show negative orientation, but at the durations of 20 and 25 minutes they come to show positive orientation, and at a duration of 30 minutes the number of positively orienting individuals tends to decrease, while that of negatively orienting individuals tends to increase, thus these number come to balance each other.

In Table 16 the changes in the magnitude of the occupied angles are shown and from this table Fig. 7 was plotted. The following facts may be seen in the figure:

TABLE 16.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 min.	86.82	111.38	114.56	85.86	119.40	123.54
3 min.	83.84	103.08	109.24	84.92	115.24	120.32
6 min.	80.06	93.80	103.74	81.38	107.94	116.56
10 min.	77.80	88.30	100.50	78.86	98.88	110.02
15 min.	76.00	83.90	97.90	78.88	93.82	104.94
20 min.	72.04	81.22	99.18	71.14	84.00	102.86
25 min.	72.16	82.14	99.98	72.70	84.78	102.08
30 min.	74.94	83.12	98.18	77.04	88.68	101.64

FIG. 7.



1. In the 5 cm. angles (broken lines), at a duration of submergence shorter than 6 minutes the orientation of the worms is negative, but at a duration longer than 10 minutes it becomes positive.

2. In the 10 cm. angles (full lines), at a duration of submergence shorter than 15 minutes the orientation of the worms is negative, but at a duration longer than 20 minutes it becomes positive.

3. In both the 5 cm. and the 10 cm. angles, the magnitude of the negative occupied angle tends to decrease retardatively according to the prolongation of the duration of submergence, but that of the positive occupied angle tends to decrease retardatively from 0 to 20 minutes and then to increase acceleratively. In consequence, the magnitude of the average occupied angle tends to decrease retardatively from 0 to 20 minutes and then to increase somewhat acceleratively, being influenced mainly by the changes in the positive occupied angle.

From Tables 15 and 16 we may conclude that when the worms are submerged in the saturated solution of strychnine and when they were not operated upon, the degree of positive orientation becomes strengthened retardatively in relation to the prolongation of the duration of submergence, while the degree of negative orientation becomes weakened retardatively, thus producing positive orientation as a whole at a duration longer than 15 or 20 minutes, though the degree of positive orientation itself becomes weakened acceleratively in relation to the prolongation from 20 to 30 minutes.

THE MOVEMENTS OF OPERATED WORMS.

Four tests were carried on from October 23 to 25 at durations of submergence of 0, 10, 20, and 30 minutes and in each test 25 worms were used individually. The temperature of the dark room was 16.0° -16.7°C.

The absolute numbers of forward and backward crawling individuals are given in Table 17. It may be seen in the table that the frequency of forward crawling tends to increase with the prolongation of the duration of submergence, while that of backward crawling tends to decrease.

The absolute numbers of positively, indifferently, and negatively phototactic individuals are shown together in Table 18. The following facts may be seen in the table:

TABLE 17.

Duration of submergence	5 cm. angles				10 cm. angles	
	Forward		Backward			
	Directly	After posterior elongation	Directly	After anterior elongation	Forward	Backward
0 min.	13	2	9	1	15	10
10 min.	14	1	10	0	15	10
20 min.	18	1	6	0	19	6
30 min.	18	2	5	0	20	5

TABLE 18.

	Duration of submergence	Positive Phototaxis			Indifferent Phototaxis			Negative Phototaxis		
		Forward crawling 0°–80°	Backward crawling 180°–100°	Sum	Forward crawling 81°–99°	Backward crawling 99°–81°	Sum	Forward crawling 100°–180°	Backward crawling 80°–0°	Sum
5 cm. angles	0 min.	11	0	11	1	1	2	3	9	12
	10 min.	12	0	12	1	3	4	2	7	9
	20 min.	13	0	13	2	2	4	4	3	7
	30 min.	15	3	18	2	1	3	3	1	4
10 cm. angles	0 min.	13	0	13	1	1	2	1	9	10
	10 min.	10	0	10	3	1	4	2	9	11
	20 min.	14	1	15	2	2	4	3	3	6
	30 min.	16	3	19	1	1	2	3	1	4

1. The number of negatively phototactic individuals tends to decrease with the prolongation of the duration of submergence, while the number of positively phototactic individuals and the difference between the positively and negatively phototactic individuals tend to increase.

2. The number of individuals which crawl forward toward the light (positively phototactic and positively orienting) tends to increase with the prolongation of the duration of submergence.

3. The number of individuals which crawl backward away from the light (negatively phototactic, but positively orienting) tends to decrease with the prolongation of the duration of submergence, while the number of individuals which crawl backward toward the light (positively phototactic, but negatively orienting) tends to increase.

From Tables 17 and 18 we may conclude that at any duration of submergence the number of forward crawling individuals is greater than that of backward crawling individuals, and that following the prolongation of the duration of submergence the number of forward crawling individuals and of positively phototactic individuals which crawl either forward or backward tends to increase, while the number of backward crawling individuals and of negatively phototactic individuals which crawl backward tends to decrease, consequently the number of positively phototactic individuals becomes greater than that of negatively phototactic individuals.

The orienting movements of the worms which were operated upon are shown in Tables 19 and 20.

In Table 19 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

1. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals (section 0°–80°) tends to decrease with the prolongation of the duration of submergence, while that of negatively orienting individuals (section 100°–180°) tends to increase.

2. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals is greater than that of negatively orienting individuals, but the difference between these numbers becomes smaller according to the prolongation of the duration of submergence.

In Table 20 the changes in the magnitude of the occupied angles are shown and from this table Fig. 8 was plotted. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, the magnitude of the positively moved angle is larger than that of the corresponding negatively moved angle, consequently the average moved angle shows the positivity of the worms which were operated upon.

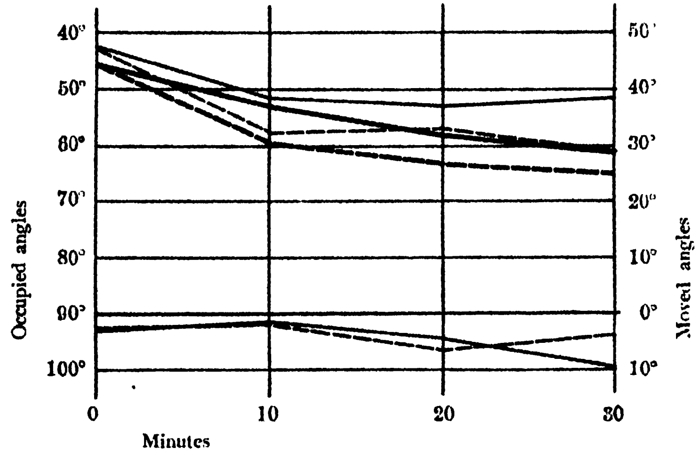
TABLE 19.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0 min.	20	2	8	22	2	1
10 min.	19	4	2	19	4	2
20 min.	16	5	4	17	4	4
30 min.	16	3	6	17	2	6

TABLE 20.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 min.	43.16	45.64	92.48	42.64	45.72	93.08
10 min.	57.52	59.68	92.16	51.72	53.12	91.40
20 min.	56.44	62.88	96.44	53.04	57.76	94.72
30 min.	60.76	65.04	94.28	51.44	61.04	99.60

FIG. 8.



2. In both the 5 cm and the 10 cm. angles, the magnitude of the positively moved angle tends to decrease retardatively with the prolongation of the duration of submergence, while the magnitude of the negatively moved angle tends to increase. In consequence the magnitude of the average moved angle shows a retardative decrease of the positivity of the worms.

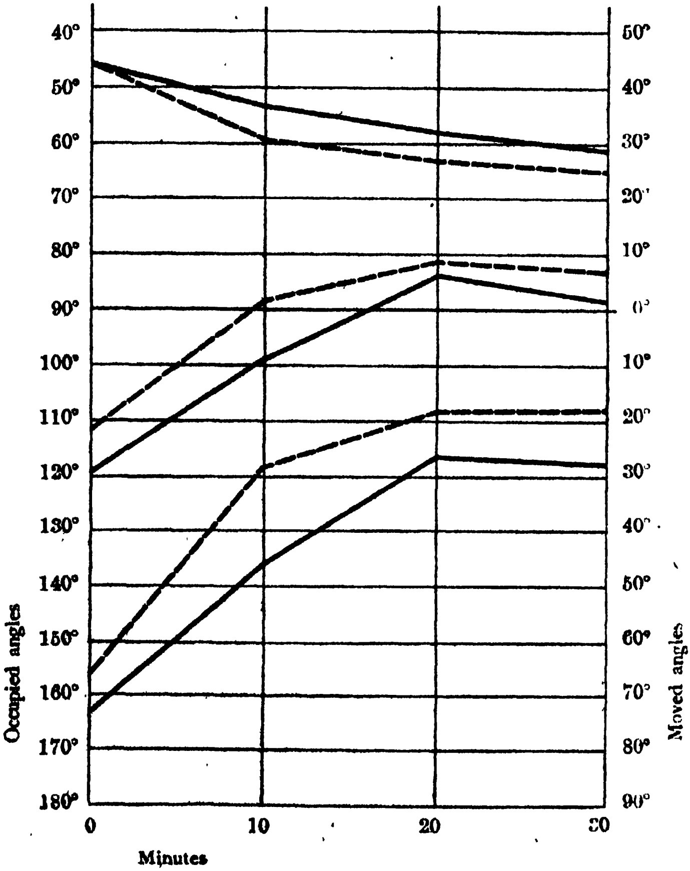
From Tables 19 and 20 we may conclude that when the worms are submerged in the saturated solution of strychnine and when they are operated upon, their degree of positive orientation becomes weaker retardatively in relation to the prolongation of the duration of submergence, and, in turn, their degree of negative orientation becomes stronger.

THE CHANGES OF NEGATIVITY IN THE BRAIN.

TABLE 21.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	P	*A*	*N*	*P*	*A*	*N*
0 min.	45.64	111.88	155.74	45.72	119.40	163.68
10 min.	59.68	88.30	118.62	53.12	98.88	135.76
20 min.	62.88	81.22	108.84	57.76	84.00	116.24
30 min.	65.04	83.12	108.08	61.04	88.68	117.64

FIG. 9.



The magnitudes of *A*, the magnitudes of *P*, and the calculated magnitudes of *N* are given together in Table 21, and from this table Fig. 9 was plotted in order to see easily the state of the changes. In the figure it may be seen that when the worms are submerged in the saturated solution of strychnine, the degree of negative orientation which is exhibited by the brain becomes weaker retardatively in relation to the prolongation of the duration of submergence.

CONCLUSIONS.

1. In the ventral nerve cord, strychnine causes a retardative weakening of the positively orienting functioning and an inhibition of backward crawling.
2. In the brain, strychnine causes a retardative weakening of the negatively orienting functioning.
3. At the beginning, the degree of negative orientation in the brain is stronger than that of positive orientation in the ventral nerve cord, but later the reverse is the case.
4. In consequence, in worms strychnine causes a retardative weakening of the degree of negative orientation or phototaxis, which changes for a retardative strengthening of the degree of positive orientation or phototaxis. The retardative change in positive orientation is due to a weakening of the positively orienting functioning of the ventral nerve cord.

Chapter IV. The effect of strychnine nitrate.

MOORE (1916). "It has been shown that strychnine has the effect of reversing the positively phototropic response of the freshwater crustacea, *Diaptomus bakeri*. If, to a dish of pond water containing some of these animals, sufficient strychnine nitrate solution be added to make the concentration 0.001%, the organisms form a collection on the side of the dish opposite the source of light. Acids and anaesthetics, on the other hand, cause the *Diaptomus* to collect on the side of the dish nearest the light, and also reverse the action of strychnine when added to the medium containing strychninized animals."

In the following experiments a 1/50 normal solution of strychnine nitrate was used.

As soon as the worms were submerged in this solution, they showed convulsion, as well as ejection of hypodermal secretion. The convulsion continued in most cases for 4–7 seconds, and in a few cases I could observe the second or even the third convulsion.

A longer duration of submergence caused a stronger contraction of circular muscle, a stronger relaxation of longitudinal muscle, and a more frequent posterior elongation.

THE MOVEMENTS OF UNOPERATED WORMS.

Seven tests were carried on from November 3 to 4 in the dark room, the temperature of which was 15.6°C., and at each test 50 worms were used after being submerged individually in the definite solution of strychnine nitrate for a duration of 0, 10, 20, 30, 40, 50, or 60 seconds.

The orienting or the phototactic movements of the worms are shown in Tables 22 and 23.

In Table 22 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

TABLE 22.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0 sec.	13	12	25	11	7	32
10 sec.	11	12	27	10	6	34
20 sec.	9	13	28	6	9	35
30 sec.	7	13	30	6	8	36
40 sec.	7	13	30	5	8	37
50 sec.	7	10	33	5	7	38
60 sec.	6	10	34	1	5	44

1. In both the 5 cm. and the 10 cm. angles, a prolongation of the duration of submergence causes a decrease of the number of positively orienting individuals (section 0°–80°) and an increase of the number of negatively orienting individuals (section 100°–180°).

2. In both the 5 cm. and the 10 cm. angles, the number of negatively orienting individuals is greater than that of positively orienting individuals.

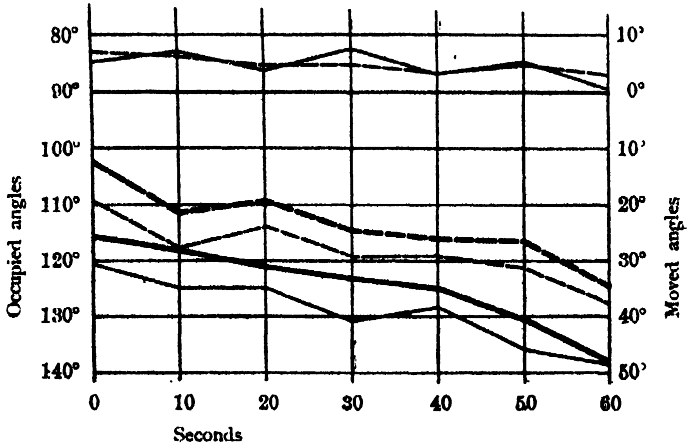
In Table 23 the changes in the magnitude of the occupied angles are shown and from this table Fig. 10 was plotted. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, the magnitude of the negatively moved angle is larger than that of the positively moved angle, consequently the average moved angle shows the negativity of the worms which were not operated upon.

TABLE 23.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 sec.	82.82	102.38	109.56	84.86	115.40	120.54
10 sec.	83.78	111.42	117.64	82.78	117.94	125.16
20 sec.	85.12	109.88	114.26	86.28	121.10	124.82
30 sec.	85.18	114.80	119.62	82.40	123.14	130.74
40 sec.	86.52	116.08	119.56	86.98	124.92	127.94
50 sec.	85.24	116.44	121.20	84.70	130.54	135.84
60 sec.	87.16	124.80	127.64	89.58	138.26	138.68

FIG. 10.



2. In both the 5 cm. and the 10 cm. angles the magnitude of the negatively moved angle tends to increase according to the prolongation of the duration of submergence, while that of the positively moved angle tends to decrease, consequently the average moved angle shows an increasing negativity of the worms.

From Tables 22 and 23 we may conclude that when the worms are submerged in the 1/50 normal solution of strychnine nitrate and when they are not operated upon, their degree of negative orientation becomes stronger with the prolongation of the duration of submergence.

THE MOVEMENTS OF OPERATED WORMS.

Seven tests were carried on from November 5 to 6 at durations of submergence of 0, 10, 20, 30, 40, 50, and 60 seconds, and in each test 25 worms were used individually. The temperature of the dark room was 14.5°C.

The absolute numbers of forward and backward crawling individuals are given in Table 24. It may be seen in the table that when the worms are not submerged in the solution of strychnine nitrate (0 second) the frequency of forward crawling is greater than that of backward crawling, but when they are submerged in the solution the frequency of forward crawling becomes smaller than that of backward crawling, and that at any duration of submergence longer than 10 seconds the frequency of forward and of backward crawling shows no notable alterations.

TABLE 24.

Duration of submergence	5 cm. angles				10 cm. angles	
	Forward		Backward			
	Directly	After posterior elongation	Directly	After anterior elongation	Forward	Backward
0 sec.	14	3	8	0	17	8
10 sec.	5	0	19	1	6	19
20 sec.	1	3	21	0	5	20
30 sec.	2	2	21	0	4	21
40 sec.	3	0	22	0	3	22
50 sec.	2	0	23	0	4	21
60 sec.	4	1	20	0	5	20

The absolute numbers of positively, indifferently, and negatively phototactic individuals are shown together in Table 25. The following facts may be seen in the table:

1. When the worms are not submerged in the solution, the number of positively phototactic individuals is slightly greater than, or nearly

equal to, the number of negatively phototactic individuals, but when they are submerged in the solution the number of negatively phototactic individuals becomes greater than that of positively phototactic individuals.

2. At any duration of submergence longer than 10 seconds, the number of positively phototactic individuals, of negatively phototactic individuals, of forward crawling individuals, and of backward crawling individuals shows no notable alterations.

TABLE 25.

	Duration of submergence	Positive Phototaxis			Indifferent Phototaxis			Negative Phototaxis		
		Forward crawling 0°–80°	Backward crawling 180°–100°	Sum	Forward crawling 81°–99°	Backward crawling 99°–81°	Sum	Forward crawling 100°–180°	Backward crawling 80°–0°	Sum
5 cm. angles	0 sec.	10	0	13	2	0	2	1	9	10
	10 sec.	4	1	5	0	6	6	1	13	14
	20 sec.	3	0	3	0	5	5	1	16	17
	30 sec.	3	4	7	1	3	4	0	14	14
	40 sec.	3	1	4	0	6	6	0	15	15
	50 sec.	1	3	4	1	7	8	0	13	13
	60 sec.	3	1	4	1	4	5	1	15	16
	Average			5.7			5.1			14.2
10 cm. angles	0 sec.	13	0	13	1	0	1	2	9	11
	10 sec.	6	1	7	0	4	4	0	14	14
	20 sec.	4	1	5	1	2	3	0	17	17
	30 sec.	3	3	6	0	2	2	1	16	17
	40 sec.	3	3	6	0	3	3	0	16	16
	50 sec.	3	2	5	0	1	1	1	18	19
	60 sec.	4	2	6	0	1	1	1	17	18
	Average			6.9			2.1			16.0

From Tables 24 and 25 we may conclude that when the worms are submerged in the 1/50 normal solution of strychnine nitrate and when they are operated upon, most of them crawl backward showing negative phototaxis, and that the duration of submergence influences little the frequency of backward crawling and the number of negatively phototactic individuals.

The orienting movements of the worms which were operated upon are shown in Tables 26 and 27.

In Table 26 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

1. In both the 5 cm. and the 10 cm. angles, the number of individuals found in section 0°–20° tends to decrease with the prolongation of the duration of submergence, while the number of individuals found in section 21°–80° tends to increase; and the sum of the numbers of individuals found in the remaining sections tends to increase from 0 to 30 seconds and then tends to decrease. These facts indicate that the worms which show strongly positive orientation (0°–20°) and which show indifferent or negative orientation (81°–180°) come to show a moderate positive orientation (21°–80°) in relation to the prolongation of the duration of submergence, though the degree of negative orientation is slightly pronounced at a duration of about 30 seconds.

TABLE 26.

Duration of submergence	5 cm. angles					10 cm. angles				
	0°–20°	21°–80°	81°–99°	100°–159°	160°–180°	0°–20°	21°–80°	81°–99°	100°–159°	160°–180°
0 sec.	9	13	2	1	0	10	12	1	2	0
10 sec.	8	16	5	1	0	8	13	3	1	0
20 sec.	2	15	6	2	0	6	14	4	1	0
30 sec.	2	15	4	4	0	5	14	2	4	0
40 sec.	2	16	6	1	0	4	15	3	3	0
50 sec.	1	20	3	0	1	5	16	1	2	1
60 sec.	1	22	1	1	0	3	18	1	3	0

2. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals (0°–80°) is greater than that of negatively orienting individuals (100°–180°).

In Table 27 the changes in the magnitude of the occupied angles are shown and from this table Fig. 11 was plotted. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, at any duration of submergence, the magnitude of the positively moved angle is larger than that of the corresponding negatively moved angle, consequently, the average moved angle shows the positivity of the worms which were operated upon.

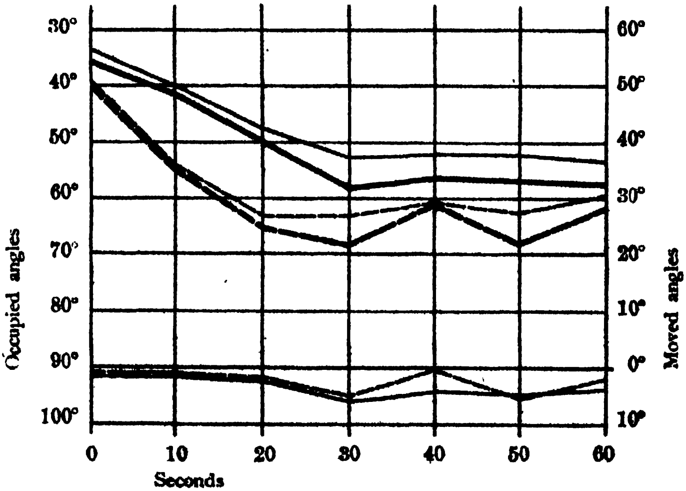
2. In both the 5 cm. and the 10 cm. angles, the magnitude of the positively moved angles decreases with the prolongation of the duration from 0 to 30 seconds, while that of the negatively moved angle in-

creases, consequently the average moved angle shows a decrease of the positivity of the worms; but when the duration is more prolonged the magnitudes of positively and negatively moved angles show no notable alterations, consequently, the average moved angle shows nearly a constant positivity of the worms at any duration of submergence.

TABLE 27.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 sec.	39.28	40.00	90.72	33.92	35.60	91.68
10 sec.	54.24	55.04	90.80	40.04	41.36	91.82
20 sec.	63.60	65.28	91.68	47.36	49.92	92.56
30 sec.	63.24	68.44	95.20	52.68	58.60	95.92
40 sec.	60.80	61.24	90.44	52.04	56.44	94.40
50 sec.	62.52	68.00	95.48	52.16	57.16	95.00
60 sec.	59.76	61.84	92.08	53.48	57.64	94.16

FIG. 11.



From Tables 26 and 27 we may conclude that when the worms are submerged in the 1/50 normal solution of strychnine nitrate and when they are operated upon, the degree of positive orientation becomes weakened in relation to the prolongation of the duration of submergence from 0 to 30 seconds, but it becomes nearly constant at durations of submergence from 30 to 60 seconds.

THE CHANGES OF NEGATIVITY IN THE BRAIN.

The mangnitudes of *P*, *A* and *N* are given together in Table 28, and from this table Fig. 12 was plotted. In the figure it may be seen that when the worms are submerged in the 1/50 normal solution of strychnine nitrate, a prolongation of duration from 0 to 20 or 30 seconds causes in the brain a decreased degree, but a prolongation above 20 or 30 seconds an increased degree, of negative orientation.

FIG. 12.

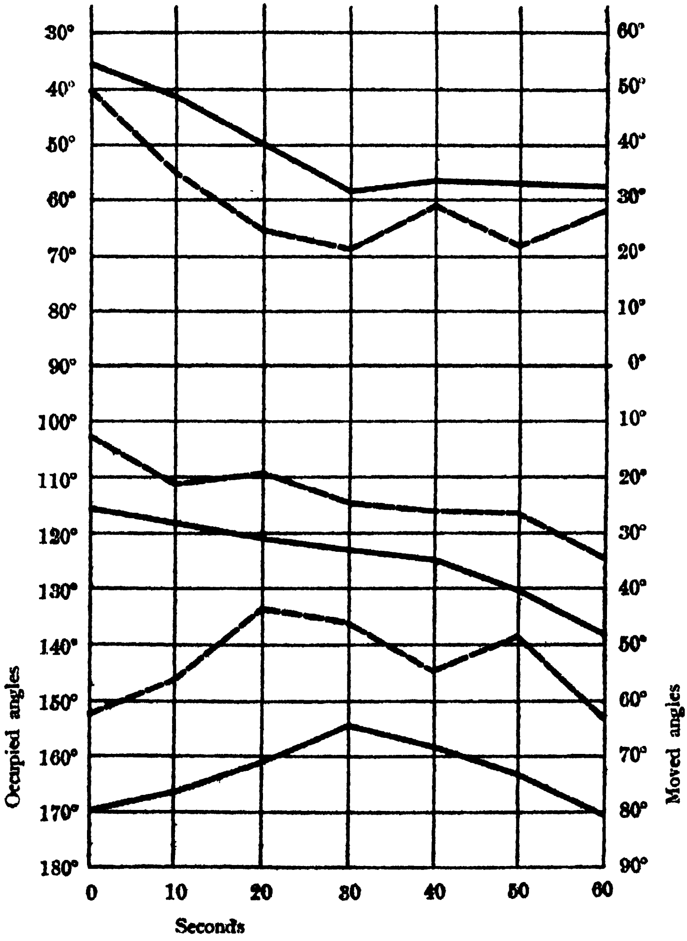


TABLE 28.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	P	*A*	*N*	*P*	*A*	*N*
0 sec.	40.00	102.38	152.38	35.60	115.40	169.80
10 sec.	55.04	111.42	146.38	41.36	117.94	166.58
20 sec.	65.28	109.38	184.10	49.92	121.10	161.18
30 sec.	68.44	114.80	136.36	58.60	123.14	154.54
40 sec	61.24	116.08	144.84	56.44	124.92	158.48
50 sec.	68.00	116.44	138.44	57.16	130.54	163.38
60 sec.	61.84	124.80	152.96	57.64	138.26	170.62

CONCLUSIONS.

1. In the ventral nerve cord, strychnine nitrate causes a weakening of the positively orienting functioning to a certain degree, and an inhibition of forward crawling.

2. In the brain, strychnine nitrate causes at first a weakening and then a strengthening of the negatively orienting functioning, but it appears not to cause a weakened inhibition of backward crawling.

3. In consequence, in worms strychnine nitrate causes a gradual strengthening of the degree of negative orientation or phototaxis, due at first to a weakening degree of positive orientation in the ventral nerve cord and then to a strengthening degree of negative orientation in the brain.

Chapter V. The effect of strychnine sulphate.

MOORE (1916). "The larvae of squid (*Loligo pealii*) are normally positively phototropic, but attempts to reverse this response by means of strychnine were unsuccessful. If 0.2 cc. of a 0.5% solution of strychnine sulfate be added to 100 cc. of sea-water containing freshly hatched squid larvae, the latter no longer form a phototropic collection either positively or negatively."

MOORE (1918). "It has been shown that a single excitation at a median point in the earthworm elicits a shortening of the body anterior and a lengthening posterior to that point. Active extension of the body is produced by contraction of the circular muscles, and by inhibition of contraction of the longitudinal muscles of the body. This is a case of reciprocal innervation (GARREY and MOORE). Further, it has been found that in the earthworm, just as in vertebrates, strychnine converts inhibition into excitation, thus causing upon stimulation a contraction of the longitudinal muscles, which results in a shortening posterior to the point of stimulation (KNOWLTON and MOORE). . . . . . Two cases have been described, that of the marine planarian *Bdelloura* and that of the starfish *Asterias forbesii*, in which strychnine (strychnine sulphate 1 : 10.000) reverse reciprocal inhibition. These facts indicate that the nervous system of these invertebrates function in a manner similar to those of the earthworm and vertebrates. Moreover, it would seem that strychnine acts upon some chemical component of the neuron which is always present in synaptic structures but which also occurs in the simpler neurons of lower forms. This fact that strychnine is without this characteristic effect on such forms as medusa and sea anemone, indicates that the nervous systems of the starfish and planarian have chemical affinities with the vertebrates which the coelenterates do not possess."

In the following experiments on *Allolobophora foetida* a 1/25 normal solution of strychnine sulphate was used.

As soon as the worms were submerged into this solution, they showed convulsion as in the case of the solution of strychnine nitrate, but in the majority of the worms the convulsion was not followed by an ejection of hypodermal secretion. The convulsion continued in most cases for 2–5 seconds and in many cases I could observe the second, or sometimes even the fourth, convulsion.

A longer duration of submergence caused in the worms an increased secretion of slime and a more sluggish locomotion, but it appeared not to cause a stronger relaxation of longitudinal muscle. When the worms were submerged into the solution for 4 minutes, most of them could not crawl for a requisite distance.

THE MOVEMENTS OF UNOPERATED WORMS.

Four tests were carried on from December 8 to 9 and at each test 50 worms were used after being submerged individually in a definite solution of strychnine sulphate for a duration of 0, 1, 2, or 3 minutes. The temperature of the dark room was 19°–21°C. This comparatively high temperature was caused by radiating heat from steam-pipes which were set up in this room.

The orienting (that is directly the phototactic) movements of the worms are shown in Table 29 and 30.

TABLE 29.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
0 min.	6	6	38	5	2	43
1 min.	6	8	36	4	5	41
2 min.	5	6	39	3	4	43
3 min.	7	7	36	6	2	42

In Table 29 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

1. In both the 5 cm. and the 10 cm. angles, any duration of submergence gives nearly the same number of positively (section 0°-80°) and of negatively orienting individuals (section 100°-180°).

2. In both the 5 cm. and the 10 cm. angles, the number of negatively orienting individuals is greater than that of positively orienting individuals.

In Table 30 the changes in the magnitude of the occupied angles are shown and from this table Fig. 13 was plotted. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, the magnitude of the negatively moved angle is larger than that of the positively moved angle, consequently the average moved angle shows the negativity of the worms.

2. In 5 cm. angles, the magnitude of the average, of the positive, or of the negative occupied angle is nearly constant at durations of submergence of 0, 1, and 2 minutes, but it becomes smaller at 3 minutes.

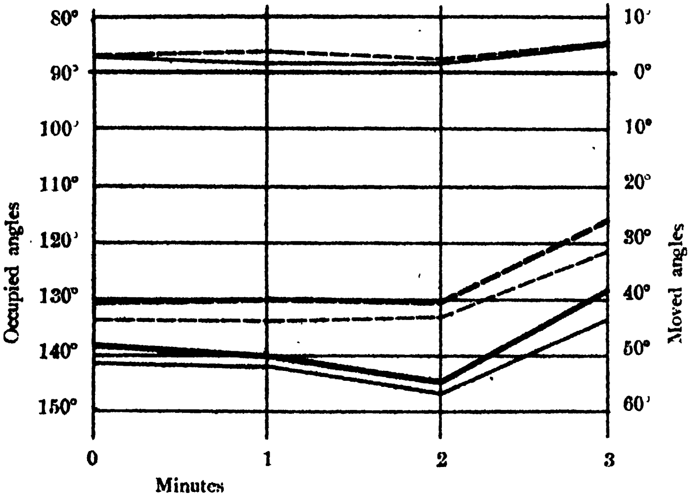
3. In 10 cm. angles, the magnitude of the average, of the positive, or of the negative occupied angle tends to increase with the prolongation of the duration from 0 to 2 minutes, but it decreases at 3 minutes.

From Tables 29 and 30 we may conclude that when the worms are submerged in the 1/25 normal solution of strychnine sulphate and

TABLE 30.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 min.	86.84	130.58	133.74	86.92	138.24	141.32
1 min.	86.17	130.10	133.93	88.48	140.15	141.67
2 min.	87.20	130.80	133.10	88.52	144.86	146.34
3 min.	84.62	115.78	121.16	84.84	128.48	133.64

FIG. 13.



when they are not operated upon, their degree of negative orientation tends to increase slightly with the prolongation of the duration of submergence from 0 to 2 minutes or remains nearly constant at any duration, but a further prolongation causes a decrease of the degree of negative orientation.

THE MOVEMENTS OF OPERATED WORMS.

Four tests were carried on on the 10th of December at durations of submergence of 0, 1, 2, and 3 minutes, and in each test 25 worms were used. The temperature in the dark room was 19°–21°C.

The absolute numbers of forward and backward crawling individuals are given in Table 31. It may be seen in the table that, in both the

5 cm. and the 10 cm. angles, at durations of submergence of 0, 1, and 2 minutes, the frequency of forward, as well as of backward, crawling is nearly constant, the former being invariably smaller than the latter; but at 3 minutes the frequency of forward crawling somewhat abruptly becomes larger than that of backward crawling.

TABLE 31.

Duration of submergence	5 cm. angles				10 cm. angles	
	Forward		Backward		Forward	Backward
	Directly	After posterior elongation	Directly	After posterior elongation		
0 min.	9	1	14	1	11	14
1 min.	9	2	12	2	11	14
2 min.	7	1	17	0	10	15
3 min.	19	2	4	0	21	4

The absolute numbers of positively, indifferently, and negatively phototactic individuals are shown together in Table 32. It may be seen in the table that, in both the 5 cm. and the 10 cm. angles, at any duration of submergence the number of positively phototactic individuals is nearly the same as, or slightly smaller than, the number of negatively phototactic individuals.

TABLE 32.

	Duration of submergence	Positive Phototaxis			Indifferent Phototaxis			Negative Phototaxis		
		Forward crawling 0°–80°	Backward crawling 180°–100°	Sum	Forward crawling 81°–99°	Backward crawling 99°–81°	Sum	Forward crawling 100°–180°	Backward crawling 80°–0°	Sum
5 cm. angles	0 min.	9	0	9	1	4	5	0	11	11
	1 min.	7	2	9	2	4	6	2	8	10
	2 min.	3	5	8	4	3	7	1	9	10
	3 min.	10	0	10	3	1	4	8	3	11
	Average			9.0			5.5			10.5
10 cm. angles	0 min.	9	0	9	2	1	3	0	13	13
	1 min.	6	4	10	2	1	3	3	9	12
	2 min.	5	5	10	2	3	5	3	7	10
	3 min.	9	1	10	4	1	5	8	2	10
	Average			9.8			4.0			11.2

The orienting movements of the worms which were operated upon are shown in Tables 33 and 34.

In Table 33 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

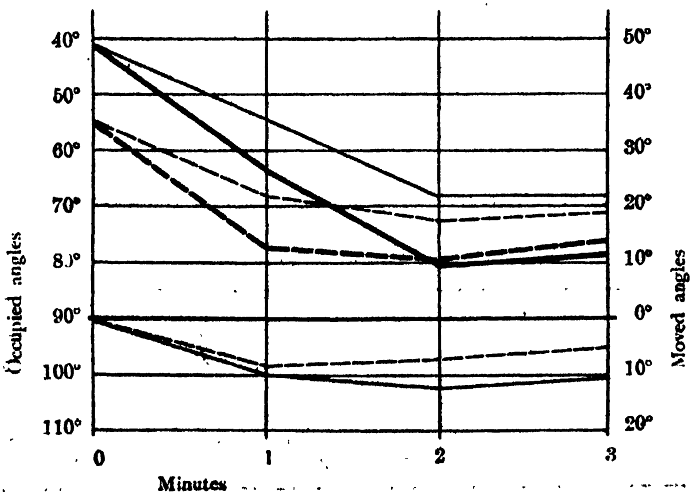
TABLE 33.

Duration of submergence	5 cm. angles			10 cm. angles		
	0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
0 min.	20	5	0	22	3	0
1 min.	13	8	4	17	1	7
2 min.	12	7	6	12	5	7
3 min.	13	8	4	11	9	5

TABLE 34.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 min.	54.88	55.04	90.16	40.80	41.08	96.28
1 min.	68.24	76.80	98.56	54.44	64.32	99.88
2 min.	72.32	79.44	97.12	68.16	80.32	102.16
3 min.	70.96	76.04	95.08	67.92	78.44	100.52

FIG. 14.



1. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals (0°–80°) tends to decrease with the prolongation of the duration of submergence, but the number of negatively orienting individuals (100°–180°) is nearly constant at durations of submergence of 1, 2, and 3 minutes.

2. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals (0°–80°) is greater than that of negatively orienting individuals (100°–180°).

In Table 34 the changes in the magnitude of the occupied angles are shown, and from this table Fig. 14 was plotted. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, at any duration of submergence, the magnitude of the positively moved angle is larger than that of the corresponding negatively moved angle, consequently, the average moved angle shows the positivity of the worms which were operated upon.

2. In both the 5 cm. and the 10 cm. angles, the magnitude of the average, of the positive, or of the negative occupied angle tends to increase retardatively with the prolongation of the duration of submergence, showing a decrease of the positivity and an increase of the negativity of the worms.

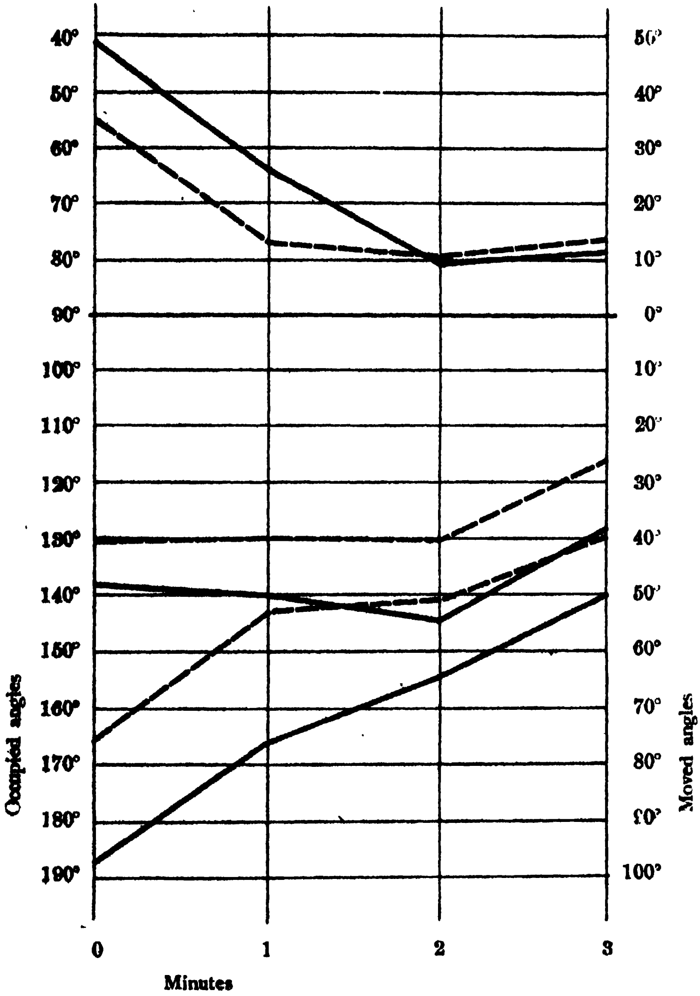
From Tables 33 and 34 we may conclude that when the worms are submerged in the 1/25 normal solution of strychnine sulphate and when they are operated upon, the degree of positive orientation becomes weakened retardatively in relation to the prolongation of the duration of submergence, while the degree of negative orientation becomes strengthened retardatively.

THE CHANGES OF NEGATIVITY IN THE BRAIN.

TABLE 35.

Duration of submergence	5 cm. angles in degrees			10 cm. angles in degrees		
	P	*A*	*N*	*P*	*A*	*N*
0 min.	55.04	130.58	165.54	41.08	138.24	187.16
1 min.	76.80	130.10	143.30	64.32	140.15	165.83
2 min.	79.44	130.80	140.86	80.32	144.86	154.54
3 min.	76.04	115.78	129.74	78.44	128.48	140.04

FIG. 15.



The magnitudes of *P*, *A*, and *N* are given together in Table 35, and from this table Fig. 15 was plotted in order to see easily the state of the changes. In this figure it may be seen that when the worms are submerged in the 1/25 normal solution of strychnine sulphate, the degree of negative orientation which is exhibited by the brain becomes weaker in relation to the prolongation of the duration of submergence.

CONCLUSIONS.

1. In the ventral nerve cord, strychnine sulphate causes a retardative weakening of the positively orienting functioning and an inhibition of backward crawling.

2. In the brain, strychine sulphate causes a weakening of the negatively orienting functioning.

3. In consequence, in worms strychnine sulphate causes at first no notable alteration, but later it causes a weakening of the degree of negative orientation or phototaxis.

Chapter VI. Summary and general conclusions.

1. In the ventral nerve cord of the worms, the degree of positively orienting functioning becomes weaker in relation to the prolongation of the duration of submergence in a solution of HCl, KCN, strychnine, strychnine sulphate, or strychnine nitrate.

2. In the brain of the worms, the degree of negatively orienting functioning becomes weaker in relation to the prolongation of the duration of submergence in a solution of HCl, KCN, strychnine, or strychnine sulphate.

3. In the brain of the worms, the degree of negatively orienting functioning becomes at first weaker and then stronger in relation to the prolongation of the duration of submergence in the solution of strychnine nitrate.

4. In consequence, from the antagonistic relation between the functionings of the brain and of the ventral nerve cord, the phototaxis in the worms shows several alterations in relation to the prolongation of the duration of submergence in a solution of HCl, KCN, strychnine, strychnine sulphate, or strychnine nitrate.

i. Strychnine causes a retardative weakening of negative phototaxis.

ii. KCN and strychnine sulphate cause an accelerative weakening of negative phototaxis.

iii. HCl causes at first a weakening and then a strengthening of negative phototaxis.

iv. Strychnine nitrate causes a strengthening of negative phototaxis.

December 25, 1925.

LITERATURE CITED.

1) Allee, W. C. 1912. An experimental analysis of the relation between physiological states and rheotaxis in isopoda. Jour. Exp. Zool., Vol. 13, pp. 269-344.

2) Allee, W. C. 1914. The ecological importance of the rheotactic reaction of stream isopods. Biol. Bull., Vol. 27, pp. 52-66.

3) Allen, G. D. 1915. Reversibility of the reactions of *Planaria dorotocephala* to a current of water. Biol. Bull., Vol. 29, pp. 111-128.

4) Bodine, J. H. 1924. Some physiological actions of cyanides. Jour. Gen. Physiol., Vol. 7, pp. 19-23.

5) Crozier, W. J. and Federighi, H. 1924. Suppression of phototropic circus movements of *Limax* by Strychnine. Jour. Gen. Physiol., Vol. 7, pp. 221-224.

6) Ewald, W. F. 1912. On artificial modification of light reactions and the influence of electrolytes on phototaxis. Jour. Exp. Zool, Vol. 13, pp. 591-612.

7) Herms, W. B. 1911. The photic reactions of sarcophagid flies, especially *Lucilia caesar* Linn. and *Calliphora vomitoria* Linn. Jour. Exp. Zool., Vol. 10, pp. 167-226.

8) Jackson, H. H. T. 1910. The control of phototactic reactions in *Hyalella* by chemicals. Jour. Comp. Neurol., Vol. 20, pp. 259-263.

9) Jennings, H. S. 1906. Modifiability in behavior. II Factors determining direction and character of movement in the earthworm. Jour. Exp. Zool., Vol. 3, pp. 435-455.

10) Kanda, S. 1918. Further studies on the geotropism of *Paramecium caudatum*. Biol. Bull., Vol. 35, pp. 108-119.

11) Kanda, S. 1919. On the reversibility of the heliotropism of *Arenicola* larvae by chemicals. Biol. Bull., Vol. 36, pp. 149-166.

12) Loeb, J. 1893. Über künstliche Umwandlung positiv heliotropischer Thiere in negativ heliotropische und umgekehrt. Pflüger's Archiv, Bd. 54, pp. 81-107.

13) Loeb, J. 1904. The control of heliotropic reactions in freshwater crustaceans by chemicals, especially CO_2. Univ. California Publications in Physiol., Vol. 2. pp. 1-3.

14) Moore, A. R. 1912 a. Concerning negative phototropism in *Daphnia pulex*. Jour. Exp. Zool., Vol. 13, pp. 573-575.

15) Moore, A. R. 1912 b. Negative phototropism in *Diaptomus* by means of strychnine. Univ. California Publications in Physiol., Vol. 4, pp. 185-186.

16) Moore, A. R. 1916. The action of strychnin on certain invertebrates. Jour. Pharmacol. and Therap., Vol. 9, pp. 167-169.

17) Moore, A. R. 1918. Reversal of reaction by means of strychnine in planarians and starfish. Jour. gen. Physiol., Vol. 1, pp. 97-100.

18) Nomura, E. 1926. Effect of light on the movements of the earthworm, *Allolobophora foetida* (Sav.). Science Reports Tohoku Imp. Univ., Biology. Vol. I, pp. 293-409.

19) Phipps, C. F. 1915. An experimental study of the behavior of amphipods with respect to light intensity, direction of rays and metabolism. Biol. Bull., Vol. 28, pp. 210-223.

20) Sasaki, K. 1924. *Allolobophora foetida* (Sav.) in North Japan. Science Reports Tohoku Imp. Univ., Biology, Vol. 1, pp. 89-90.

21) Wodsedalek, J. E. 1911. Phototactic reactions and their reversal in the may-fly nymphs *Heptagenia interpunctata* (Say). Biol. Bull., Vol. 21, pp. 265-271.

The Influence of Coastal Waves on the Shell-Development in *Littorina sitchana* PHIL.1)

BY

EKITARO NOMURA.

According to BUMPUS2) "the American type of *Littorina littorea* L., when compared with the European type, is more elongated, lighter in weight, more bulky, and the color markings are less pronounced."

Rikuoku Wan (Bay) is divided by the peninsula of Natsudomari into Aomori Wan and Nobechi Wan (Fig. 1). Asamushi is located on the western side of the peninsula near the base and Asadokoro on the eastern side about 6 miles from Asamushi. Asamushi is exposed

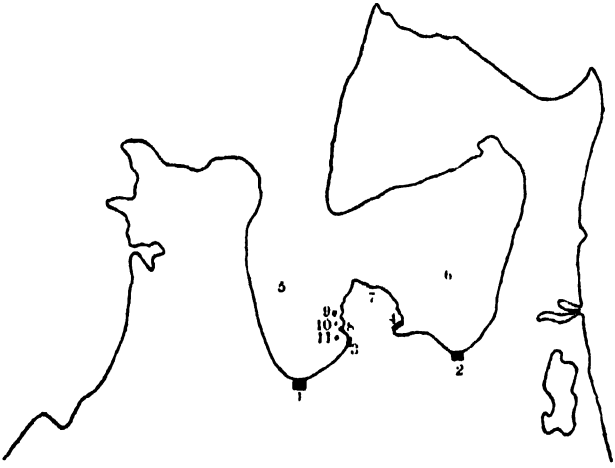


Fig. 1. Coast line of Aomori-Ken. 1. Aomori, 2. Nobechi, 3. Asamushi, 4. Asadokoro, 5. Aomori Wan, 6. Nobechi Wan, 7. Natsudomari peninsula, 8. Asamushi Marine Biological Station, 9. Mourajima, 10. Gomijima, 11. Yunoshima.

---

1) A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

2) BUMPUS, H. C. 1898. The variations and mutations of the introduced *Littorina*. Zool. Bull., Vol. 1, pp. 247-259.

to the north and west wind, while Asadokoro is exposed only to the east wind. Consequently the sea at Asadokoro is generally calmer throughout the year than the sea at Asamushi.

Littorina sitchana PHIL. is a periwinkle which is common among the stones and on the rocks along the shore of the northern part of Hondo. We also find the species at both the localities of Asamushi and Asadokoro. If coastal waves influence the shell-development, the shells from these localities must show some variations. For the purpose of investigating the variations two ratios, b/a and d/c (Fig. 2), were determined.

A larger value of b/a denotes that the shell is more flattened.

A larger value of d/c denotes that the shell is more elongated.

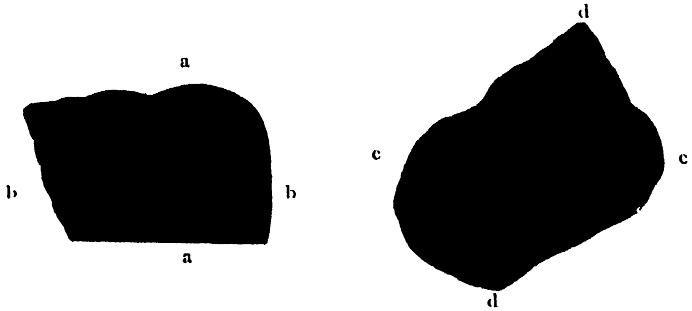


Fig. 2. Shells to illustrate the points at which measurements were taken. Magnified.

Size of shells in mm.	Asamushi Number of individuals studied	b/a	d/c	Asadokoro Number of individuals studied	b/a	d/c
4.0- 4.9	50	1.220	0.950	—	—	—
5.0- 5.9	111	1.248	0.970	—	—	—
6.0- 6.9	218	1.304	0.980	34	1.252	0.982
7.0- 7.9	131	1.352	1.014	93	1.258	0.996
8.0- 8.9	51	1.410	1.060	177	1.310	1.008
9.0- 9.9	19	1.420	1.060	140	1.342	1.016
10.0-10.9	—	—	—	56	1.390	1.032

In the table, the size of the shells was determined by the height aa. The table reveals the following facts:

1. Similarly at both localities, a larger size of the shell tends to increase the value of b/a as well as of d/c, showing that with the

growth of the body the shell becomes more flattened and more elongated.

2. The values of b/a and d/c are larger at Asamushi than at Asadokoro. The fact shows that the shell is more flattened and more elongated at Asamushi than at Asadokoro.

From the above relations it appears to me that coastal waves may be one factor causing variations in the shells of *Littorina sitchana*.

Here I wish to express my sincere thanks to Miss MATSU FUKUSHIMA for her kind assistance given and faithfulness shown during the progress of the investigation.

October 15, 1925.

An Application of $a=kb^x$ in Expressing the Growth Relation in the Freshwater Bivalve, *Sphaerium heterodon* PILS.

BY

EKITARO NOMURA.

It was already known that the equation $a=kb^x$ can be applied in expressing the growth curves in several cases, when a and b denote measurements. It was the object of the present investigation to test the applicability of the equation in expressing the growth relation between the height and the width, as well as between the weight and the width, in *Sphaerium heterodon* PILS. and, if the equation were applicable, to determine the value of k, as well as of x, for this definite species.

The specimens of *Sphaerium heterodon* PILS. were collected from a city gutter in Komegafukuro, Sendai, and were kindly indentified by Mr. SHICHIHEI NOMURA in the Geological Institute, Tohoku Imperial University. The measurements were taken from 498 specimens by Miss MATSU FUKUSHIMA, my trustworthy assistant.

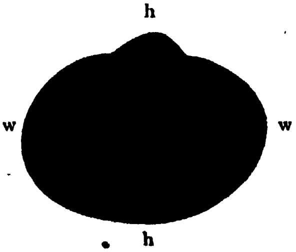


Fig. 1. A shell to illustrate the points at which measurements were taken. Much magnified. h-h height, w-w width.

THE RELATION BETWEEN THE HEIGHT AND THE WIDTH.

In applying the equation $a=kb^x$ to express the relation between the height, which is a, and the width, which is b, if we give different values to x, we obtain values of k correspondingly different from the actual measurements given in Table 1.

Among several values of x, when we put 1.25 for x at two decimal places, we obtain 0.355 as the average value of k at three decimal places; when 1.26 for x, 0.348 for k; and when 1.27 for x, 0.341 for k.

So $$\text{Height}=0.355\times\text{Width}^{1.25} \qquad \text{(I)}$$

$$\text{Height}=0.348\times\text{Width}^{1.26} \qquad \text{(II)}$$

$$\text{Height} = 0.341 \times \text{Width}^{1.27} \qquad \text{(III)}$$

If we assume (I), the total sum of the differences between the average and the calculated heights is 1.24 mm. and the largest difference is 0.19 mm.; in 27 out of 35 cases the difference is below 0.05 mm., in 7 it is from 0.05 to 0.10 mm., and in 1 it is above 0.10 mm.

If we assume (II), the total sum of the differences between the average and the calculated heights is 1.15 mm. and the largest difference is 0.18 mm.; in 27 out of 35 cases the difference is below 0.05 mm., in 5 it is from 0.05 to 0.10 mm., and in 3 it is above 0.10 mm.

TABLE 1.

Width in mm.	Number of shells studied	Average height in mm.	Height in mm., where $x=1.25$ $k=0.255$.	Difference in mm.	Height in mm., where $x=1.26$, $k=0.348$.	Difference in mm.	Height in mm., where $x=1.27$, $k=0.341$.	Difference in mm.
6.1	2	3.40	3.40	0.00	3.40	0.00	3.39	0.01
6.2	3	3.57	3.47	0.10	3.47	0.10	3.46	0.11
6.3	5	3.56	3.54	0.02	3.54	0.02	3.53	0.03
6.4	11	3.63	3.61	0.02	3.61	0.02	3.60	0.03
6.5	7	3.67	3.68	0.01	3.68	0.01	3.67	0.00
6.6	6	3.67	3.76	0.09	3.75	0.08	3.75	0.08
6.7	10	3.82	3.83	0.01	3.82	0.00	3.82	0.00
6.8	16	3.89	3.90	0.01	3.89	0.00	3.89	0.00
6.9	19	3.92	3.97	0.05	3.97	0.05	3.96	0.04
7.0	14	4.05	4.04	0.01	4.04	0.01	4.04	0.01
7.1	11	4.09	4.11	0.02	4.11	0.02	4.11	0.02
7.2	19	4.18	4.19	0.01	4.19	0.01	4.18	0.00
7.3	19	4.23	4.26	0.03	4.26	0.03	4.26	0.03
7.4	28	4.33	4.33	0.00	4.33	0.00	4.33	0.00
7.5	15	4.39	4.41	0.02	4.41	0.02	4.41	0.02
7.6	20	4.45	4.48	0.03	4.48	0.03	4.48	0.03
7.7	20	4.52	4.55	0.03	4.56	0.04	4.56	0.04
7.8	25	4.62	4.63	0.01	4.63	0.01	4.63	0.01
7.9	23	4.68	4.70	0.02	4.70	0.02	4.71	0.03
8.0	25	4.79	4.77	0.02	4.78	0.01	4.78	0.01
8.1	23	4.87	4.85	0.02	4.85	0.02	4.86	0.01
8.2	24	4.93	4.93	0.00	4.93	0.00	4.93	0.00
8.3	25	5.04	5.00	0.04	5.01	0.03	5.01	0.03
8.4	20	5.01	5.08	0.07	5.08	0.07	5.09	0.08
8.5	16	5.34	5.15	0.19	5.16	0.18	5.17	0.17
8.6	8	5.25	5.23	0.02	5.24	0.01	5.24	0.01
8.7	18	5.31	5.30	0.01	5.31	0.00	5.32	0.01
8.8	15	5.39	5.38	0.01	5.39	0.00	5.40	0.01
8.9	10	5.51	5.46	0.05	5.47	0.04	5.48	0.03
9.0	14	5.53	5.53	0.00	5.54	0.01	5.55	0.02
9.1	16	5.51	5.61	0.10	5.62	0.11	5.63	0.12
9.2	2	5.75	5.69	0.06	5.70	0.05	5.71	0.04
9.3	3	5.80	5.77	0.03	5.78	0.02	5.79	0.01
9.4	6	5.87	5.84	0.03	5.86	0.01	5.87	0.00
9.5	5	5.82	5.92	0.10	5.94	0.12	5.95	0.13
Sum of the differences				1.24		1.15		1.17

If we assume (III), the total sum of the differences between the average and the calculated heights is 1.17 mm. and the largest difference

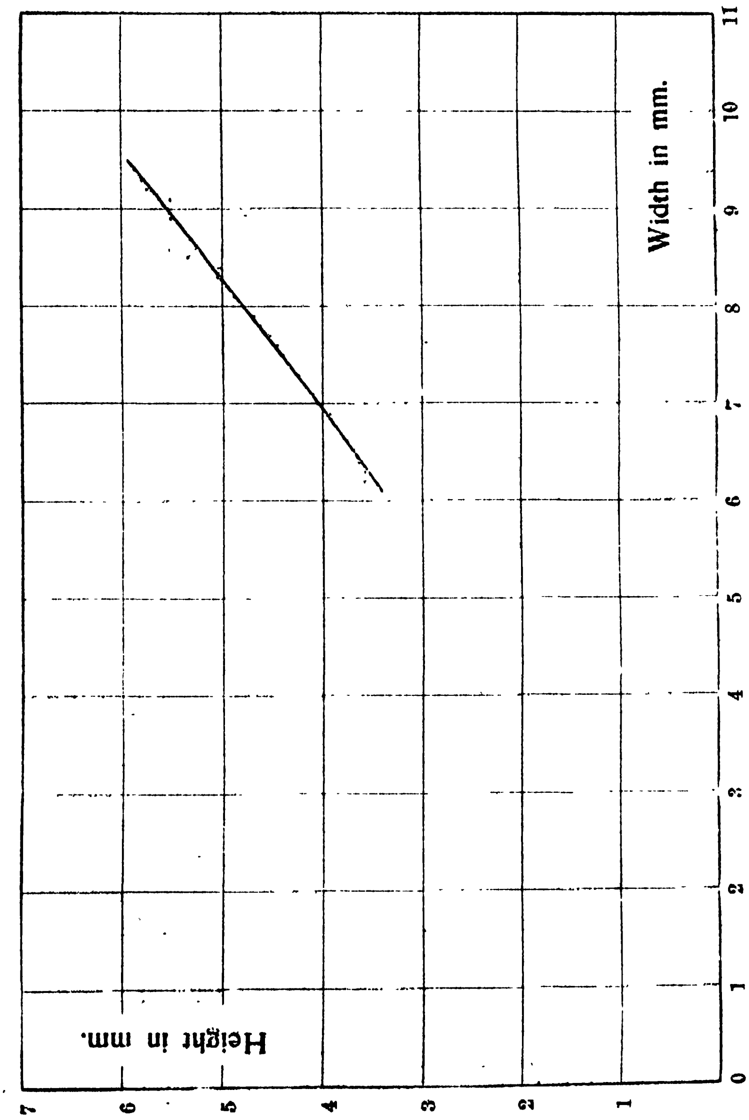


FIG. 2.

is 0.17 mm.; in 29 out of 35 cases the difference is below 0.05 mm., in 2 it is from 0.05 to 0.10 mm., but in 4 it is above 0.10 mm.

As seen in the above, each equation may be applicable in expressing the growth relation between the height and the width, but (II) seems to me to be preferable as the one for *Sphaerium heterodon* PILS., because this equation gives the least value for the total sum of differences, which ought to mean that the relation which is determined by the equation is in most intimate coincidence with the natural relation expressed by the actual measurements.

In Fig. 2 the relation which is expressed by (II) alone is shown.

Form the relation $\text{Height}=0.348\times\text{Width}^{1.26}$,
we obtain the relation $\text{Width}=2.312\times\text{Height}^{0.79}$.

THE RELATION BETWEEN THE WEIGHT AND THE WIDTH.

We obtain the three following equations as the ones which express the relation between the weight and the width in nearest approximation with the natural relation shown by the actual measurements which are given in Table 2.

$$\text{Weight}=0.150\times\text{Width}^{3.21} \qquad \text{(IV)}$$
$$\text{Weight}=0.147\times\text{Width}^{3.22} \qquad \text{(V)}$$
$$\text{Weight}=0.144\times\text{Width}^{3.23} \qquad \text{(VI)}$$

If we assume (IV), the total sum of the differences between the average and the calculated weights is 69.6 mgm. and the largest difference is 9.5 mgm.; in 14 out of 35 cases the difference is below 1.00 mm., in 19 it is from 1.00 to 5.00 mgm., and in 2 it is above 5.00 mgm.

If we assume (V), the total sum of the differences between the average and the calculated weights is 69.1 mgm. and the largest difference is 9.3 mgm.; in 13 out of 35 cases the difference is below 1.00 mgm., in 20 it is from 1.00 to 5.00 mgm., and in 2 it is above 5.00 mgm.

If we assume (VI), the total sum of the differences between the average and the calculated weights is 69.3 mgm. and the largest difference is 9.2 mgm.; in 12 out of 35 cases the difference is below 1.00 mgm., in 21 it is from 1.00 to 5.00 mgm., and in 2 it is above 5.00 mgm.

TABLE 2.

Width in mm.	Number of shells studied	Average weight in mgm.	Weight in mgm., where $x=3.21$, $k=0.150$.	Difference in mgm.	Weight in mgm., where $x=3.22$, $k=0.147$.	Difference in mgm.	Height in mgm., where $x=3.28$, $k=0.144$.	Difference in mgm.
6.1	2	45.7	49.8	4.1	49.7	4.0	49.6	3.9
6.2	3	53.7	52.4	1.3	52.3	1.4	52.2	1.5
6.3	5	54.4	55.2	0.8	55.1	0.7	55.0	0.6
6.4	11	57.3	58.1	0.8	58.0	0.7	57.9	0.6
6.5	7	60.3	61.0	0.7	60.9	0.6	60.8	0.5
6.6	6	61.8	64.1	2.3	64.0	2.2	63.9	2.1
6.7	10	65.5	67.3	1.8	67.2	1.7	67.1	1.6
6.8	16	70.5	70.5	0.0	70.5	0.0	70.4	0.1
6.9	19	71.8	73.9	2.1	73.9	2.1	73.8	2.0
7.0	14	76.5	77.4	0.9	77.4	0.9	77.3	0.8
7.1	11	80.2	81.0	0.8	81.0	0.8	80.9	0.7
7.2	19	86.3	84.8	1.5	84.7	1.6	84.6	1.7
7.3	19	88.3	88.6	0.3	88.6	0.3	88.5	0.2
7.4	23	95.0	92.5	2.5	92.5	2.5	92.5	2.5
7.5	15	97.4	96.6	0.8	96.6	0.8	96.6	0.8
7.6	20	101.3	100.8	0.5	100.8	0.5	100.8	0.5
7.7	20	103.2	105.1	1.9	105.1	1.9	105.1	1.9
7.8	25	110.1	109.6	0.5	109.6	0.5	109.6	0.5
7.9	23	115.5	114.2	1.3	114.2	1.3	114.2	1.3
8.0	25	122.1	118.9	3.2	118.9	3.2	118.9	3.2
8.1	23	127.6	123.7	3.9	123.8	3.8	123.8	3.8
8.2	24	132.0	128.7	3.3	128.8	3.2	128.8	3.2
8.3	25	136.9	133.8	3.1	133.9	3.0	134.0	2.9
8.4	20	138.2	139.0	0.8	139.1	0.9	139.2	1.0
8.5	16	153.9	144.4	9.5	144.6	9.3	144.7	9.2
8.6	8	153.0	149.9	3.1	150.1	2.9	150.2	2.8
8.7	18	153.8	155.6	1.8	155.8	2.0	156.0	2.2
8.8	15	168.6	161.4	7.2	161.7	6.9	161.9	6.7
8.9	10	169.6	167.4	2.2	167.6	2.0	167.9	1.7
9.0	14	176.6	173.4	3.2	173.8	2.8	174.0	2.6
9.1	16	178.6	179.7	1.1	180.1	1.4	180.3	1.7
9.2	2	186.1	186.2	0.1	186.5	0.4	186.8	0.7
9.3	3	193.1	192.8	0.3	193.2	0.1	193.4	0.3
9.4	6	198.7	199.5	0.8	199.9	1.2	200.3	1.6
9.5	5	205.3	206.4	1.1	206.8	1.5	207.2	1.9
Sum of the differences				69.6		69.1		69.3

As seen in the above, each equation may be applied to express the growth relation between the weight and the width, but (V) seems to me to be preferable as the one for *Sphaerium heterodon* PILS. in the same sense as that mentioned in the relation between the height and the width.

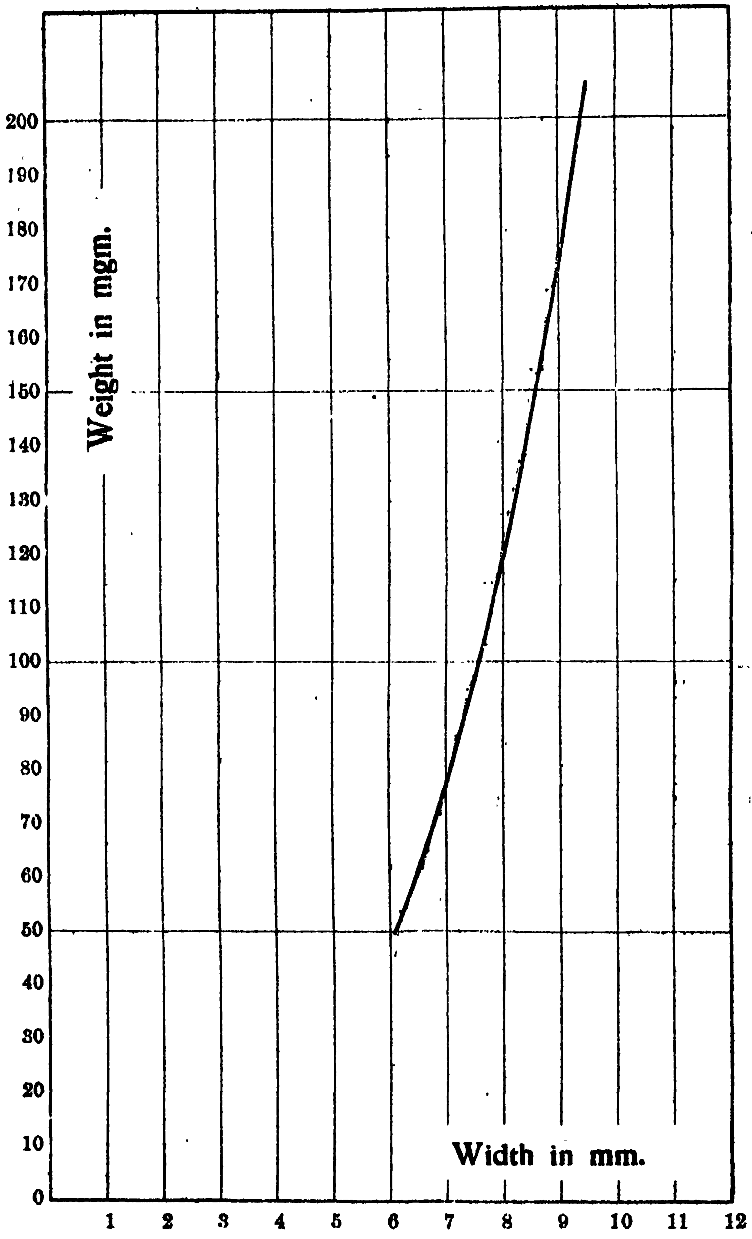
Fig. 3 is plotted from the expression by (V).

From the relation $\text{Weight} = 0.147 \times \text{Width}^{3.22}$,

we obtain the relation $\text{Width} = 1.814 \times \text{Weight}^{0.31}$.

November 20, 1925.

FIG. 3.


Weight in mgm.
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Width in mm.
1
2
3
4
5
6
7
8
9
10
11
12

Further Studies on the Applicability of $a=kb^x$ in Expressing the Growth Relations in Molluscan Shells.

BY

EKITARO NOMURA.

(Four Plates)

We have learned in my preceding paper[1] the applicability of $a=kb^x$ in expressing the growth relation between the height a and the width b in *Sphaerium heterodon* PILS. and how to determine the value of x as well as of k. The object of the present investigation was to test further the applicability of the equation to several molluscan shells and to know, if possible, the biological meaning of k as well as of x.

The four following species were studied:

Littorina sitchana PHIL.
Sphaerium heterodon PILS.
Limnaea japonica JAY
Viviparus japonicus var. *iwakawai* PILS.

Before taking up the subject of this paper I wish to express my heartfelt thanks to Miss MATSU FUKUSHIMA for her faithful and tireless assistance given during the progress of the investigation.

THE GROWTH RELATION BETWEEN THE HEIGHT AND THE WIDTH IN *Littorina sitchana* PHIL.

(Tables 1–7 and Plate 1)

The specimens were collected at the following different localities:

1. Yunoshima, a small island located in Aomori Wan about 1000 meters north-west of Asamushi[2] and about 800 meters south-west of

---

[1] NOMURA, E. 1926 a. An application of $a=kb^x$ in expressing the growth relation in the freshwater bivalve, *Sphaerium heterodon* PILS. Science Reports Tohoku Imp. Univ., Biology, Vol. 2, pp. 57-62.

[2] Ditto. 1926 b. The influence of coastal waves on the shell-development in *Littorina sitchana* PHIL. Ibid, Vol 2, pp. 53-55.

the Asamushi Marine Biological Station.

2. Gomijima, also a small island located about 1200 meters north of Yunoshima and about 500 meters north of the Biological Station.

3. Asadokoro, a sea-shore village which faces Nobechi Wan.

4. Watanoha, a fishery harbour near Ishinomaki about 20 miles north-east of Sendai.

TABLE 1.

Littorina (Yunoshima, Asamushi).

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
5.0	2	4.20	4.38	8.9	18	6.79	6.71
5.3	2	4.70	4.57	9.0	20	6.84	6.76
5.5	1	4.60	4.70	9.1	10	6.84	6.82
5.7	1	4.90	4.82	9.2	8	7.06	6.87
5.8	1	4.60	4.88	9.3	12	7.08	6.93
5.9	5	4.88	4.95	9.4	10	7.13	6.98
6.0	1	4.90	5.01	9.5	14	7.16	7.04
6.1	4	4.93	5.07	9.6	6	7.05	7.09
6.2	5	5.04	5.13	9.7	8	7.13	7.14
6.3	3	5.13	5.19	9.8	10	7.21	7.20
6.4	4	5.18	5.25	9.9	9	7.31	7.26
6.5	2	5.30	5.31	10.0	19	7.46	7.31
6.6	5	5.42	5.37	10.1	6	7.28	7.36
6.7	4	5.48	5.43	10.2	10	7.43	7.42
6.8	11	5.46	5.49	10.3	12	7.41	7.47
6.9	9	5.58	5.55	10.4	4	7.50	7.52
7.0	21	5.60	5.61	10.5	9	7.57	7.58
7.1	11	5.71	5.67	10.6	6	7.57	7.63
7.2	9	5.76	5.73	10.7	9	7.64	7.68
7.3	15	5.75	5.79	10.9	3	7.60	7.79
7.4	8	5.85	5.85	11.0	5	7.86	7.84
7.5	13	5.95	5.91	11.1	7	7.97	7.90
7.6	7	6.14	5.97	11.2	4	8.12	7.95
7.7	19	6.20	6.02	11.3	1	7.90	8.00
7.8	11	6.15	6.08	11.4	2	8.00	8.05
7.9	18	6.10	6.14	11.5	5	8.16	8.11
8.0	28	6.28	6.20	11.6	2	7.95	8.16
8.1	5	6.26	6.25	11.7	4	8.30	8.21
8.2	20	6.13	6.31	11.8	2	8.20	8.26
8.3	17	6.38	6.37	11.9	4	8.12	8.31
8.4	14	6.45	6.42	12.0	2	8.30	8.36
8.5	29	6.60	6.48	12.1	1	8.60	8.42
8.6	6	6.58	6.54	12.4	2	8.50	8.57
8.7	13	6.67	6.59	12.6	1	8.80	8.67
8.8	17	6.70	6.65	—	—	—	—

TABLE 2.

Littorina (Asadokoro)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
7.2	1	6.00	6.12	11.9	16	8.86	8.88
7.8	2	6.35	6.50	12.0	22	8.94	8.94
7.9	2	6.30	6.56	12.1	13	8.97	8.99
8.0	5	6.42	6.62	12.2	13	9.14	9.05
8.1	1	6.90	6.68	12.3	9	9.18	9.10
8.2	6	6.70	6.74	12.4	7	9.16	9.16
8.3	7	6.70	6.80	12.5	11	9.22	9.21
8.4	8	6.80	6.86	12.6	12	9.14	9.26
8.5	5	6.90	6.92	12.7	6	9.28	9.32
8.6	9	6.92	6.98	12.8	10	9.42	9.37
8.7	8	7.18	7.04	12.9	8	9.44	9.43
8.8	5	7.24	7.10	13.0	10	9.49	9.48
8.9	3	7.27	7.16	13.1	9	9.46	9.54
9.0	8	7.26	7.22	13.2	8	9.64	9.59
9.1	9	7.46	7.28	13.3	11	9.53	9.64
9.2	6	7.35	7.34	13.4	6	9.98	9.70
9.3	6	7.47	7.40	13.5	6	9.75	9.75
9.4	10	7.54	7.46	13.6	8	9.74	9.80
9.5	7	7.37	7.52	13.7	6	9.78	9.86
9.6	10	7.57	7.58	13.8	4	9.95	9.91
9.7	3	7.43	7.64	13.9	4	10.05	9.96
9.8	2	7.65	7.69	14.0	5	10.26	10.02
9.9	4	7.80	7.75	14.1	4	10.20	10.07
10.0	7	8.01	7.81	14.2	6	10.08	10.12
10.1	6	7.90	7.87	14.3	3	10.07	10.18
10.2	9	8.21	7.92	14.4	2	10.30	10.23
10.3	5	8.24	7.98	14.5	5	10.08	10.28
10.4	7	8.19	8.04	14.6	3	10.27	10.33
10.5	5	8.16	8.10	14.7	3	10.17	10.38
10.6	12	8.27	8.15	14.8	3	10.20	10.44
10.7	9	8.54	8.21	14.9	2	10.15	10.49
10.8	9	8.23	8.27	15.0	3	10.53	10.54
10.9	7	8.31	8.32	15.1	2	10.77	10.59
11.0	17	8.59	8.38	15.2	3	10.50	10.64
11.1	12	8.58	8.44	15.3	3	10.40	10.70
11.2	12	8.48	8.49	15.4	2	10.85	10.75
11.3	9	8.67	8.55	15.6	1	10.40	10.85
11.4	11	8.71	8.60	15.7	1	11.30	10.90
11.5	15	8.77	8.66	15.8	1	10.60	10.95
11.6	11	8.64	8.71	15.9	3	11.03	11.01
11.7	18	8.63	8.77	16.5	1	11.00	11.31
11.8	10	8.81	8.83	—	—	—	—

TABLE 3.

Littorina (Yunoshima, Asamushi)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
7.0	6	5.63	5.63	9.6	2	7.10	7.11
7.2	4	5.80	5.75	9.7	7	7.23	7.17
7.3	2	5.65	5.81	9.8	11	7.17	7.22
7.4	10	5.84	5.87	9.9	8	7.18	7.28
7.5	4	5.85	5.92	10.0	21	7.30	7.33
7.6	5	6.02	5.98	10.1	4	7.30	7.39
7.7	8	6.14	6.04	10.2	8	7.49	7.44
7.8	8	6.16	6.10	10.3	2	7.70	7.49
7.9	6	6.32	6.16	10.4	4	7.45	7.55
8.0	10	6.31	6.22	10.5	3	7.33	7.60
8.1	7	6.36	6.28	10.6	2	7.55	7.65
8.2	6	6.38	6.33	10.7	3	7.67	7.71
8.3	9	6.39	6.39	10.8	7	7.73	7.76
8.4	12	6.43	6.44	10.9	7	7.80	7.81
8.5	7	6.49	6.50	11.0	5	7.86	7.87
8.6	6	6.52	6.56	11.1	1	8.00	7.92
8.7	14	6.61	6.61	11.2	5	8.02	7.97
8.8	6	6.58	6.67	11.3	4	7.88	8.03
8.9	6	6.68	6.72	11.4	3	8.03	8.08
9.0	16	6.69	6.78	11.5	4	8.23	8.13
9.1	8	6.81	6.84	11.8	1	8.40	8.29
9.2	2	7.00	6.89	11.9	1	8.40	8.35
9.3	9	6.97	6.95	12.0	2	8.20	8.39
9.4	4	7.10	7.00	12.1	1	8.30	8.44
9.5	11	7.04	7.06	13.2	1	9.20	9.00

TABLE 4.

Littorina (Asadokoro)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
8.5	3	6.87	6.85	9.9	3	8.13	7.70
8.7	6	6.87	6.97	10.0	13	7.81	7.76
8.8	5	6.98	7.03	10.1	8	7.83	7.82
8.9	7	7.07	7.09	10.2	5	7.98	7.88
9.0	7	7.14	7.16	10.3	4	7.95	7.94
9.1	4	7.25	7.22	10.4	7	8.19	8.00
9.2	6	7.10	7.28	10.5	6	8.20	8.06
9.3	6	7.17	7.34	10.6	7	8.17	8.12
9.4	9	7.47	7.40	10.7	5	8.18	8.18
9.5	8	7.47	7.46	10.8	8	8.24	8.23
9.6	3	7.27	7.52	10.9	12	8.28	8.29
9.7	6	7.67	7.58	11.0	24	8.33	8.35
9.8	2	7.55	7.64	11.1	6	8.47	8.41

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
11.2	16	8.46	8.47	13.5	7	9.79	9.78
11.3	11	8.52	8.53	13.6	1	10.00	9.83
11.4	12	8.66	8.58	13.7	7	9.91	9.89
11.5	14	8.64	8.64	13.8	2	9.95	9.95
11.6	11	8.72	8.70	13.9	5	9.88	10.00
11.7	7	8.63	8.76	14.0	8	10.06	10.06
11.8	7	8.69	8.82	14.2	5	10.02	10.17
11.9	11	8.93	8.87	14.3	1	10.00	10.22
12.0	22	8.90	8.93	14.4	4	10.10	10.28
12.1	12	9.10	8.99	14.5	4	10.15	10.33
12.2	10	9.04	9.05	14.6	3	10.50	10.39
12.3	17	9.01	9.10	14.7	2	10.45	10.44
12.4	7	9.16	9.16	14.8	1	10.00	10.50
12.5	14	9.29	9.22	14.9	2	10.55	10.55
12.6	9	9.32	9.27	15.0	4	10.75	10.60
12.7	10	9.22	9.33	15.1	1	10.90	10.66
12.8	11	9.32	9.39	15.3	1	10.60	10.77
12.9	2	9.30	9.44	15.6	1	11.00	10.93
13.0	6	9.52	9.50	15.7	1	10.70	10.98
13.1	4	9.53	9.55	15.8	2	11.35	11.04
13.2	7	9.69	9.61	16.0	8	11.10	11.15
13.3	5	9.62	9.67	16.7	1	11.60	11.52
13.4	4	9.65	9.72	16.8	1	11.60	11.58

TABLE 5.

Littorina (Watanoha)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
3.7	1	3.00	3.52	8.4	3	6.63	6.62
4.5	1	3.40	4.09	8.5	1	6.40	6.68
6.0	1	5.20	5.11	8.6	2	6.85	6.74
6.3	1	5.70	5.31	8.7	8	6.78	6.80
6.5	2	5.06	5.43	8.8	6	6.87	6.86
6.6	2	4.90	5.50	8.9	3	6.83	6.92
6.7	1	5.30	5.56	9.0	11	6.97	6.98
6.8	1	5.40	5.63	9.1	11	7.15	7.04
7.0	1	6.00	5.75	9.2	4	7.20	7.10
7.2	1	6.30	5.88	9.3	5	7.30	7.16
7.3	1	5.50	5.94	9.4	6	7.43	7.22
7.4	2	5.80	6.01	9.5	5	7.38	7.28
7.6	1	5.90	6.13	9.6	3	7.33	7.34
7.7	2	6.20	6.19	9.7	4	7.38	7.40
7.8	1	6.00	6.25	9.8	6	7.67	7.46
7.9	1	6.20	6.32	9.9	3	7.67	7.51
8.0	3	6.38	6.38	10.0	12	7.78	7.57
8.2	8	6.55	6.50	10.1	7	7.81	7.63
8.3	5	6.38	6.56	10.2	5	7.98	7.69

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
10.3	5	8.20	7.75	13.1	8	9.55	9.32
10.4	4	8.20	7.80	13.2	9	9.63	9.38
10.5	6	7.97	7.86	13.3	5	9.82	9.43
10.6	8	8.06	7.92	13.4	6	9.97	9.49
10.7	6	8.15	7.98	13.5	18	9.87	9.54
10.8	9	8.12	8.03	13.6	9	9.80	9.60
10.9	2	8.25	8.09	13.7	12	9.77	9.65
11.0	8	8.29	8.15	13.8	2	9.95	9.70
11.1	7	8.47	8.21	13.9	14	9.89	9.76
11.2	5	8.44	8.26	14.0	5	9.80	9.81
11.3	5	8.80	8.32	14.1	3	9.90	9.87
11.4	8	8.54	8.38	14.2	5	10.14	9.92
11.5	2	8.85	8.43	14.3	8	10.00	9.97
11.6	6	8.73	8.49	14.4	2	10.05	10.03
11.7	5	8.76	8.55	14.5	2	10.00	10.08
11.8	8	8.66	8.60	14.6	5	10.00	10.13
11.9	9	8.77	8.66	14.7	2	10.20	10.19
12.0	16	9.04	8.71	14.8	4	10.28	10.24
12.1	7	9.03	8.77	14.9	6	10.37	10.29
12.2	9	9.17	8.83	15.0	10	10.33	10.35
12.3	14	9.11	8.88	15.2	2	10.00	10.45
12.4	6	9.30	8.94	15.3	4	10.63	10.51
12.5	8	9.18	8.99	15.4	4	9.90	10.56
12.6	6	9,22	9.05	15.5	2	10.10	10.61
12.7	6	9.25	9.10	15.6	3	10.60	10.66
12.8	10	9.50	9.16	15.8	1	10.70	10.77
12.9	1	8.90	9.21	16.8	1	10.70	11.30
13.0	22	9.54	9.27	—	—	—	—

TABLE 6.

Littorina (Gomijima, Asamushi)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
8.9	1	7.00	6.86	10.4	19	7.86	7.68
9.0	1	7.00	6.92	10.5	24	7.76	7.73
9.1	1	7.00	6.97	10.6	20	7.92	7.78
9.3	4	7.10	7.08	10.7	19	8.05	7.84
9.4	3	7.10	7.14	10.8	24	8.00	7.89
9.5	5	7.26	7.19	10.9	28	7.98	7.94
9.6	11	7.36	7.25	11.0	36	8.08	7.99
9.7	4	7.45	7.30	11.1	14	8.03	8.05
9.8	4	7.58	7.35	11.2	25	8.17	8.10
9,9	6	7.53	7.41	11.3	16	8.21	8.15
10.0	27	7.63	7.46	11.4	28	8.31	8.20
10.1	10	7.73	7.52	11.5	17	8.29	8.25
10,2	12	7.79	7.57	11.6	12	8.37	8.30
10.3	18	7.75	7.62	11.7	15	8.47	8.36

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
11.8	10	8.40	8.41	12.9	2	8.75	8.96
11.9	11	8.29	8.46	13.0	3	8.68	9.01
12.0	13	8.34	8.51	13.1	1	8.00	9.06
12.1	15	8.41	8.56	13.2	2	9.05	9.11
12.2	6	8.60	8.61	13.4	2	9.00	9.21
12.3	6	8.83	8.66	13.5	2	8.55	9.26
12.5	2	8.95	8.76	13.6	1	9.30	9.31
12.6	8	9.01	8.81	14.1	1	9.80	9.56
12.8	7	8.91	8.91	—	—	—	—

EQUATIONS FOR SPECIMENS FROM YUNOSHIMA AND ASADOKORO.

(First determination)

We have already studied the difference in the relation between the height and the width of specimens from the two localities of Asamushi and Asadokoro.[1] It was the purpose of the present investigation to learn what the difference would be when $a=kb^c$ was applied to express the growth relation of this species.

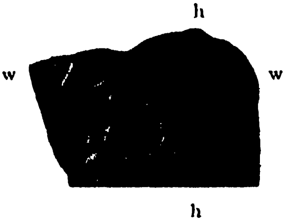


Fig. 1. *Littorina sitchana* PHIL. to illustrate the points at which measurements were taken. Magnified. h-h height, w-w width.

The measurements of 586 specimens from Yunoshima and 573 specimens from Asadokoro were taken by Miss MATSU FUKUSHIMA, and after calculation we obtained

Height $= 1.330 \times \text{Width}^{0.74}$ for Yunoshima specimens (Table 1), and Height $= 1.421 \times \text{Width}^{0.74}$ for Asadokoro specimens (Table 2).

According to the equations, the difference between the growth relations at these different localities is nothing but the difference between the numerical values of k. This result appeared to me very interesting and caused me to undertake further tests of the relation in specimens from the same, as well as from different, localities.

EQUATIONS FOR SPECIMENS FROM YUNOSHIMA AND ASADOKORO.

(Second determination)

The measurements of 303 specimens from Yunoshima and 471 specimens from Asadokoro were taken by Miss MATSU FUKUSHIMA, and after calculation we obtained

---

[1] NOMURA, b. *l. c.*

$$\text{Height} = 1.333 \times \text{Width}^{0.74} \text{ for Yunoshima specimens (Table 3),}$$

and $\text{Height} = 1.318 \times \text{Width}^{0.77}$ for Asadokoro specimens (Table 4).

The equation for the specimens from Yunoshima shows an intimate coincidence with that which was already obtained, while the equation for the specimens from Asadokoro gives a different value for k as well as for x. But if we tentatively make the value of x 0.74, even the specimens from Asadokoro give the equation, $\text{Height} = 1.417 \times \text{Width}^{0.74}$, and this shows also an intimate coincidence with that which was already obtained.

EQUATION FOR SPECIMENS FROM WATANOHA.

The measurements of 496 specimens were taken by Miss MATSU FUKUSHIMA. In this determination smaller and larger specimens showed a resemblance to those from Yunoshima, while specimens of middle-size showed rather a near resemblance to those from Asadokoro, consequently the specimens appeared to give a growth relation which might be expressed by an equation of a different kind, but after calculation we obtained

$$\text{Height} = 1.286 \times \text{Width}^{0.77} \qquad \text{(Table 5)}$$

as the only exponential equation for the specimens from Watanoha.

EQUATION FOR SPECIMENS FROM GOMIJIMA.

The measurements of 496 specimens were taken by Miss MATSU FUKUSHIMA, and after calculation we obtained

$$\text{Height} = 1.422 \times \text{Width}^{0.73} \qquad \text{(Table 6)}$$

as the only equation for the specimens from Gomijima.

THE BIOLOGICAL MEANING OF x AS WELL AS OF k.

We have obtained in the above, for each set of specimens, only one equation which expresses a mean relation between the height and the width. As shown in Tables 1–6, however, we actually have numerous individual measurements which do not agree with the mean relation, and naturally the value of k is the average of different values of individual k under a fixed value of x, which is determined for the set of specimens. It follows, then, that in individual equations the value of x is constant, but the value of k may differ according to different individuals.

The six following equations were obtained in the above studies:

$\text{Height} = 1.421 \times \text{Width}^{0.74}$ for Asadokoro specimens I,
$\text{Height} = 1.318 \times \text{Width}^{0.77}$ for Asadokoro specimens II,
$\text{Height} = 1.286 \times \text{Width}^{0.77}$ for Watanoha specimens,
$\text{Height} = 1.422 \times \text{Width}^{0.72}$ for Gomijima specimens,
$\text{Height} = 1.333 \times \text{Width}^{0.74}$ for Yunoshima specimens II,
$\text{Height} = 1.330 \times \text{Width}^{0.74}$ for Yunoshima specimens I.

In these equations the difference between the largest and the smallest values of k is 0.136, while that of x is only 0.05. This fact may probably be taken to show that the species, even from different localities, gives the same value for x all through, but it may give different values for k according to different localities. In my opinion the different values of x which were obtained are due to the small number of specimens which were studied.

If we allow ourselves to think along this line, it may be said that in the equation $a=kb^x$ the value of x is invariable in a definite species, while the value of k is variable according to individuals, as well as to locality. Then it follows that x relates to the specific consistency and k to the individual or the local variation of specimens. Following the gross relations, I wish to consider x as the specific exponent and k as the local constant.

DETERMINATION OF THE VALUE OF THE SPECIFIC EXPONENT.

As already seen we could not obtain an unique value of x for *Littorina sitchana* PHIL. due probably to the small number of specimens which were studied, consequently, at present, I may mention only that the value of the specific exponent of this species is probably 0.74 ± 0.03. I am of the opinion that we need not determine the value of the specific exponent so sharply, because a species or variety will not everlastingly be identified in utilizing it.

DETERMINATION OF THE RATIOS BETWEEN THE LOCAL CONSTANTS.

Let us suppose that naturally the value of the specific exponent can not be different for a definite species, *Littorina sitchana* PHIL., even though different values for x are actually found in the equations. If the probable value of the specific exponent is determined, then the corresponding value of the local constant becomes determined.

In assuming $x=0.72$, the following relations are obtained:

$\text{Height}=1.488\times\text{Width}^{0.72}$ for Asadokoro specimens I,
$\text{Height}=1.483\times\text{Width}^{0.72}$ for Asadokoro specimens II,
$\text{Height}=1.450\times\text{Width}^{0.72}$ for Watanoha specimens,
$\text{Height}=1.422\times\text{Width}^{0.72}$ for Gomijima specimens,
$\text{Height}=1.395\times\text{Width}^{0.72}$ for Yunoshima specimens II,
$\text{Height}=1.393\times\text{Width}^{0.72}$ for Yunoshima specimens I.

In assuming $x=0.74$, the following relations are obtained:

$\text{Height}=1.421\times\text{Width}^{0.74}$ for Asadokoro specimens I,
$\text{Height}=1.417\times\text{Width}^{0.74}$ for Asadokoro specimens II,
$\text{Height}=1.385\times\text{Width}^{0.74}$ for Watanoha specimens,
$\text{Height}=1.358\times\text{Width}^{0.74}$ for Gomijima specimens,
$\text{Height}=1.333\times\text{Width}^{0.74}$ for Yunoshima specimens II,
$\text{Height}=1.330\times\text{Width}^{0.74}$ for Yunoshima specimens I.

In assuming $x=0.77$, the following relations are obtained:

$\text{Height}=1.320\times\text{Width}^{0.77}$ for Asadokoro specimens I,
$\text{Height}=1.318\times\text{Width}^{0.77}$ for Asadokoro specimens II,
$\text{Height}=1.286\times\text{Width}^{0.77}$ for Watanoha specimens,
$\text{Height}=1.262\times\text{Width}^{0.77}$ for Gomijima specimens,
$\text{Height}=1.240\times\text{Width}^{0.77}$ for Yunoshima specimens II,
$\text{Height}=1.236\times\text{Width}^{0.77}$ for Yunoshima specimens I.

In Table 7 the values of k which were determined from the determination of the value of x and the ratios between the values of k are given together. In the calculation of the ratios, for convenience, the value of k from the first set of Asadokoro specimens was taken as the unit. It may be seen in the table that the ratio between the values of k at two localities is constant, even though the absolute values of k are different following the difference of the value of x.

TABLE 7.

Littorina sitchana PHIL.

		$x=0.72$		$x=0.74$		$x=0.77$	
		Value of k	Ratio between k	Value of k	Ratio between k	Value of k	Ratio between k
Asadokoro	I	1.488	1.000	1.421	1.000	1.320	1.000
	II	1.488	0.997	1.417	0.997	1.318	0.998
Watanoha		1.450	0.974	1.385	0.975	1.286	0.974
Gomijima		1.422	0.956	1.358	0.956	1.262	0.956
Yunoshima	II	1.395	0.938	1.333	0.938	1.240	0.939
	I	1.393	0.936	1.330	0.936	1.236	0.936

If we assume this relation just stated, it follows then that when we fix any probable value for the specific exponent for a definite species, *Littorina sitchana* PHIL., the different values of the local constant determine the degrees of the local variations. Moreover, from the fact that at either Asadokoro or Yunoshima the difference between the ratios from the different sets of specimens hardly surpassed 0.003, the determination of the ratios sppears to me very reliable.

THE GROWTH RELATION BETWEEN THE HEIGHT AND THE WIDTH IN *Sphaerium heterodon* PILS.

(Tables 8 and 9, and Plate 2)

We have studied already that 498 specimens from a city gutter at Komegafukuro, Sendai, showed the relation[1]

$$\text{Height}=0.348\times\text{Width}^{1.26}\qquad\text{(Table 8).}$$

In order to test this relation again, 1025 specimens were collected by Mr. FUTOSHI ONODERA, Miss MATSU FUKUSHIMA, and myself from an irrigating gutter at Kita-Rokubancho, Sendai, and the measurements were taken by Miss MATSU FUKUSHIMA in the same way as in the specimens from Komegafukuro.

After calculation the relation

$$\text{Height}=0.337\times\text{Width}^{1.26}\qquad\text{(Table 9)}$$

---

[1] NOMURA, a. *l. c.*

TABLE 8.

Sphaerium (Komegafukuro, Sendai)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
6.1	2	3.40	3.40	7.9	23	4.68	4.70
6.2	3	3.57	3.47	8.0	25	4.79	5.78
6.3	5	3.56	3.54	8.1	23	4.87	4.85
6.4	11	3.63	3.61	8.2	24	4.93	4.93
6.5	7	3.67	3.68	8.3	25	5.04	5.01
6.6	6	3.67	3.75	8.4	20	5.01	5.08
6.7	10	3.82	3.82	8.5	16	5.34	5.16
6.8	16	3.89	3.89	8.6	8	5.25	5.24
6.9	19	3.92	3.97	8.7	18	5.31	5.31
7.0	14	4.05	4.04	8.8	15	5.39	5.39
7.1	11	4.09	4.11	8.9	10	5.51	5.47
7.2	19	4.18	4.19	9.0	14	5.53	5.54
7.3	19	4.23	4.26	9.1	16	5.61	5.62
7.4	23	4.33	4.33	9.2	2	5.75	5.70
7.5	15	4.39	4.41	9.3	3	5.80	5.78
7.6	20	4.45	4.48	9.4	6	5.87	5.86
7.7	20	4.52	4.56	9.5	5	5.82	5.94
7.8	25	4.62	4.63	—	—	—	—

TABLE 9.

Sphaerium (Kita-Rokubancho, Sendai)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
6.0	29	3.16	3.11	8.3	30	4.59	4.65
6.1	23	3.10	3.17	8.4	30	4.66	4.72
6.2	30	3.29	3.24	8.5	30	4.82	4.79
6.3	30	3.39	3.30	8.6	30	4.78	4.86
6.4	30	3.43	3.37	8.7	30	4.91	4.93
6.5	30	3.47	3.43	8.8	25	4.96	5.00
6.6	30	3.55	3.50	8.9	18	5.16	5.07
6.7	30	3.57	3.57	9.0	27	5.20	5.14
6.8	30	3.64	3.63	9.1	25	5.17	5.21
6.9	30	3.73	3.70	9.2	21	5.40	5.28
7.0	30	3.73	3.76	9.3	14	5.35	5.35
7.1	30	3.83	3.83	9.4	11	5.51	5.42
7.2	30	3.87	3.90	9.5	17	5.55	5.50
7.3	30	3.95	3.96	9.6	9	5.63	5.57
7.4	18	4.08	4.03	9.7	12	5.62	5.64
7.5	30	4.13	4.10	9.8	11	5.72	5.71
7.6	30	4.22	4.17	10.0	11	5.85	5.86
7.7	30	4.18	4.24	10.1	3	6.00	5.93
7.8	29	4.22	4.30	10.2	5	5.96	6.00
7.9	19	4.34	4.37	10.3	3	5.93	6.08
8.0	30	4.41	4.44	10.4	2	6.00	6.15
8.1	30	4.56	4.51	10.5	2	6.10	6.22
8.2	30	4.54	4.58	10.7	1	6.50	6.37

was obtained for the specimens from Kita-Rokubancho with the same specific exponent but with a different local constant from the specimens from Komegafukuro.

THE GROWTH RELATION BETWEEN THE HEIGHT AND THE WIDTH IN *Limnaea japonica* JAY.

(Tables 10–12, and Plate 3)

1261 specimens were collected by Mr. KUNIO ITO from a pool at Mukaiyama, Sendai, and 413 specimens were collected by Mr. FUTOSHI ONODERA, Miss. MATSU FUKUSHIMA, and myself from an irrigating gutter at Odawara, Sendai. The species was kindly identified by Prof. SANJI HOZAWA, and the measurements were taken by Miss MATSU FUKUSHIMA.

After calculation we obtained the following relations:

Height$=0.537 \times$ Width$^{0.99}$ for Mukaiyama specimens (Table 10),
and Height$=0.466 \times$ Width$^{1.02}$ for Odawara specimens (Table 11).

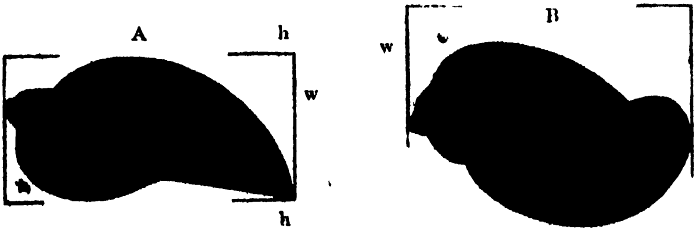


Fig. 2. *Limnaea japonica* JAY to illustrate the relation between the height and the width. Magnified. A. side view, B. dorsal view, h–h. height, w–w. width.

DETERMINATION OF THE VALUE OF THE SPECIFIC EXPONENT.

If we do not need to determine the value of the specific exponent so sharply, 1.00 seems to me to be most practical for *Limnaea japonica* JAY.

DETERMINATION OF THE RATIO BETWEEN THE LOCAL CONSTANTS.

In Table 12 the values of the local constant k, which were

TABLE 10.

Limnaea (Mukaiyama, Sendai)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
5.0	16	2.68	2.64	9.7	17	5.11	5.09
5.1	6	2.38	2.69	9.8	10	5.14	5.14
5.2	10	2.80	2.75	9.9	10	5.20	5.20
5.3	7	2.80	2.80	10.0	32	5.25	5.25
5.4	18	2.84	2.85	10.1	9	5.30	5.30
5.5	10	2.94	2.90	10.2	14	5.45	5.35
5.6	16	2.97	2.96	10.3	19	5.34	5.40
5.7	17	3.00	3.01	10.4	15	5.44	5.46
5.8	16	3.03	3.06	10.5	23	5.36	5.51
5.9	19	3.06	3.11	10.6	10	5.61	5.56
6.0	36	3.19	3.16	10.7	10	5.61	5.61
6.1	15	3.23	3.22	10.8	17	5.60	5.67
6.2	16	3.24	3.27	10.9	10	5.64	5.72
6.3	19	3.31	3.32	11.0	23	5.78	5.77
6.4	32	3.35	3.37	11.1	7	5.69	5.82
6.5	24	3.48	3.43	11.2	13	5.82	5.87
6.6	14	3.45	3.48	11.3	8	5.74	5.92
6.7	26	3.57	3.53	11.4	8	6.01	5.98
6.8	19	3.58	3.58	11.5	15	5.95	6.03
6.9	23	3.72	3.63	11.6	6	6.23	6.08
7.0	37	3.69	3.68	11.7	7	6.17	6.13
7.1	13	3.77	3.74	11.8	9	6.16	6.18
7.2	25	3.87	3.79	11.9	3	6.37	6.23
7.3	33	3.83	3.84	12.0	11	6.22	6.29
7.4	21	3.90	3.89	12.1	5	6.24	6.34
7.5	30	3.83	3.95	12.2	4	6.30	6.39
7.6	10	4.07	4.00	12.3	16	6.39	6.44
7.7	19	4.02	4.05	12.4	8	6.53	6.49
7.8	29	4.06	4.10	12.5	7	6.37	6.54
7.9	22	4.17	4.16	12.6	4	6.48	6.59
8.0	29	4.18	4.21	12.7	1	6.50	6.65
8.1	10	4.29	4.26	12.8	5	6.58	6.70
8.2	16	4.30	4.31	12.9	2	6.55	6.75
8.3	25	4.39	4.36	13.0	1	6.40	6.80
8.4	19	4.38	4.42	13.1	3	6.80	6.86
8.5	16	4.48	4.47	13.2	8	6.60	6.91
8.6	14	4.59	4.52	13.3	3	6.97	6.96
8.7	17	4.66	4.57	13.5	2	7.05	7.06
8.8	15	4.55	4.62	13.7	1	7.30	7.17
8.9	14	4.63	4.68	13.8	1	7.00	7.22
9.0	29	4.76	4.73	14.0	2	7.35	7.32
9.1	14	4.80	4.78	14.1	1	7.60	7.37
9.2	18	4.84	4.83	14.3	1	7.90	7.48
9.3	9	4.87	4.88	14.6	1	8.00	7.63
9.4	15	4.93	4.94	14.7	1	7.90	7.68
9.5	12	5.04	4.99	15.2	1	8.30	7.94
9.6	12	5.04	5.04	—	—	—	—

TABLE 11.

Limnaea (Odawara, Sendai)

Width in mm.	No of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
7.3	1	3.50	3.54	12.5	7	5.99	6.13
7.4	1	3.60	3.59	12.6	4	6.18	6.18
7.6	2	3.80	3.69	12.7	6	6.10	6.23
7.7	1	3.60	3.74	12.8	4	6.80	6.28
7.8	1	3.10	3.79	12.9	1	6.20	6.33
8.0	3	4.07	3.89	13.0	4	6.30	6.38
8.1	1	3.80	3.94	13.1	1	6.50	6.43
8.2	1	4.10	3.99	13.2	4	6.53	6.48
8.3	1	4.00	4.03	13.3	4	6.68	6.53
8.4	1	4.10	4.08	13.4	4	6.80	6.58
8.5	1	4.10	4.13	13.5	6	6.85	6.63
8.6	2	4.20	4.18	13.6	4	6.58	6.68
8.7	3	4.20	4.23	13.7	3	6.73	6.73
8.8	2	4.50	4.28	13.8	4	6.73	6.78
8.9	2	4.50	4.33	14.0	3	7.00	6.88
9.0	2	4.55	4.38	14.1	2	7.15	6.93
9.1	2	4.35	4.43	14.2	2	6.50	6.98
9.2	3	4.83	4.48	14.3	1	7.40	7.03
9.3	2	4.55	4.53	14.5	1	6.80	7.13
9.4	2	4.80	4.58	14.6	1	7.10	7.18
9.5	4	4.58	4.63	14.8	3	7.43	7.28
9.6	3	4.93	4.68	14.9	1	7.00	7.33
9.7	3	4.70	4.73	15.0	2	7.05	7.38
9.8	7	4.87	4.78	15.1	3	7.53	7.43
9.9	6	4.80	4.83	15.2	3	7.77	7.48
10.0	12	4.89	4.88	15.4	2	7.55	7.58
10.1	5	4.92	4.93	15.5	7	7.64	7.63
10.2	5	4.88	4.98	15.6	2	7.80	7.68
10.3	5	5.04	5.03	15.8	3	7.67	7.78
10.4	8	5.08	5.08	16.0	2	8.00	7.88
10.5	9	5.10	5.13	16.1	1	8.10	7.93
10.6	4	5.00	5.18	16.2	2	8.50	7.98
10.7	5	5.16	5.23	16.3	2	8.50	8.03
10.8	6	5.22	5.28	16.5	1	8.20	8.13
10.9	3	5.33	5.33	16.6	1	8.00	8.18
11.0	10	5.17	5.28	16.7	1	8.70	8.23
11.1	8	5.29	5.43	16.8	1	8.50	8.28
11.2	6	5.47	5.48	16.9	2	8.90	8.33
11.3	6	5.45	5.53	17.0	3	8.07	8.38
11.4	8	5.65	5.58	17.1	1	8.50	8.43
11.5	13	5.41	5.63	17.2	2	8.55	8.48
11.6	6	5.65	5.68	17.3	7	8.89	8.53
11.7	11	5.67	5.73	17.4	3	8.90	8.58
11.8	11	5.72	5.78	17.5	4	8.70	8.63
11.9	9	5.77	5.83	17.7	1	8.50	8.74
12.0	6	5.78	5.88	17.8	4	8.93	8.79
12.1	3	5.80	5.93	17.9	3	9.10	8.84
12.2	3	5.87	5.98	18.0	1	8.70	8.89
12.3	8	5.98	6.03	18.1	1	9.50	8.94
12.4	6	6.15	6.08	18.2	1	9.00	8.99

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
18.3	2	9.25	9.04	19.7	4	9.58	9.74
18.5	3	9.40	9.14	19.8	1	9.70	9.80
18.6	8	9.43	9.19	20.0	4	9.93	9.90
18.7	2	9.20	9.24	20.1	1	10.00	9.94
18.8	3	9.20	9.29	20.5	4	10.23	10.15
18.9	1	9.20	9.34	20.6	1	10.20	10.20
19.1	1	9.10	9.44	20.7	2	10.20	10.25
19.2	8	9.43	9.49	21.0	1	10.70	10.40
19.4	1	9.50	9.59	21.4	1	10.40	10.60
19.5	2	10.10	9.64	22.1	1	10.90	10.96
19.6	2	10.10	9.69	22.5	1	10.80	11.16

determined from the determination of the value of the specific exponent x and the ratios between the values of k are given together. In the calculation of the ratios, for convenience, the value of k from the Mukaiyama specimens was taken as the unit. It may be seen in this table that, as in Table 7, the ratio between the values for k at the two localities is constant even with a different value for x.

TABLE 12.

Limnaea japonica JAY

	$x=0.99$		$x=1.00$		$x=1.02$	
	Value of k	Ratio between k	Value of k	Ratio between k	Value of k	Ratio between k
Mukaiyama	0.537	1.000	0.524	1.000	0.498	1.000
Odawara	0.502	0.935	0.490	0.935	0.466	0.936

THE GROWTH RELATION BETWEEN THE HEIGHT AND THE WIDTH IN *Viviparus japonicus* VAR. *iwakawai* PILS.

(Tables 13–15 and Plate 4.)

214 specimens were collected from a paddy-field near Hanamaki, Iwate-Ken, by Mr. KOZO MIKAMI, a business-man who is much interested in scientific investigation, and 360 specimens were collected from a city pond in Sendai by Mr. FUTOSHI ONODERA. The species was kindly identified by Mr. SHICHIHEI NOMURA, and the measurements were taken by Mr. FUTOSHI ONODERA.

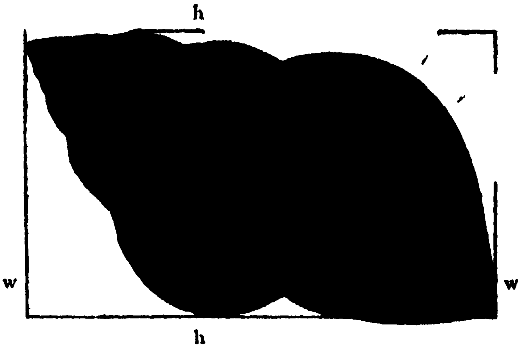


Fig. 3. *Viviparus japonicus* var. *iwakawai* PILS. to illustrate the relation between the height and the width. Magnified. h–h. height, w–w. width.

DETERMINATION OF THE VALUE OF THE SPECIFIC EXPONENT.

1.00 seems to be most practical for *Viviparus japonicus* var. *iwakawai* PILS.

DETERMINATION OF THE RATIO BETWEEN THE LOCAL CONSTANTS.

In Table 15 the values of the local constant and the ratios between

TABLE 13.

Viviparus (Hanamaki, Iwate-Ken)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
18.5	1	12.80	13.05	26.8	1	19.20	18.77
20.2	1	14.00	14.23	27.0	1	19.00	18.91
21.2	1	14.60	14.92	27.1	2	18.95	18.98
21.6	1	15.20	15.19	27.2	1	19.50	19.04
22.0	1	15.50	15.47	27.5	2	19.30	19.25
22.7	2	15.70	15.95	27.6	1	19.20	19.32
23.1	1	16.10	16.22	27.7	3	19.47	19.39
24.8	1	16.20	17.40	27.8	1	18.80	19.46
25.1	1	16.70	17.60	27.9	2	19.65	19.52
25.9	1	18.50	18.15	28.0	1	20.00	19.59
26.1	1	18.00	18.29	28.1	1	19.60	19.66
26.4	1	19.30	18.50	28.2	5	20.06	19.73

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
28.3	1	20.60	19.80	33.4	3	23.44	23.29
28.4	1	20.60	19.87	33.5	5	23.30	23.36
28.5	1	19.50	19.94	33.6	1	24.30	23.43
28.6	1	20.00	20.01	33.7	4	23.43	23.49
28.7	1	19.40	20.08	33.8	2	23.50	23.56
28.8	1	20.00	20.14	33.9	3	23.40	23.63
29.1	2	21.05	20.35	34.1	3	23.43	23.77
29.2	1	21.00	20.42	34.2	2	24.10	23.84
29.3	2	20.90	20.49	34.3	2	24.35	23.91
29.4	2	20.40	20.56	34.4	2	24.15	23.97
29.5	4	20.20	20.62	34.5	2	24.05	24.04
29.6	1	21.00	20.69	34.6	1	23.70	24.11
29.7	5	20.78	20.76	34.7	2	24.20	24.18
29.8	1	21.10	20.82	34.8	2	24.25	24.25
30.0	3	21.00	20.96	34.9	4	24.38	24.32
30.2	2	22.10	21.10	35.0	4	24.45	24.38
30.3	3	20.43	21.17	35.1	1	25.60	24.45
30.4	3	21.17	21.24	35.4	4	24.63	24.66
30.5	1	21.30	21.31	35.6	1	24.20	24.80
30.6	4	21.35	21.38	35.9	2	24.80	25.00
30.7	2	21.70	21.45	36.1	2	25.15	25.13
30.8	1	22.70	21.51	36.2	1	24.80	25.20
30.9	1	21.40	21.58	36.3	4	25.73	25.27
31.0	4	21.40	21.65	36.8	1	25.50	25.61
31.1	1	22.00	21.72	36.9	2	25.90	25.68
31.2	5	22.00	21.79	37.1	1	25.70	25.81
31.4	1	22.20	21.92	37.2	1	25.50	25.88
31.5	3	22.30	21.99	37.3	2	26.65	25.95
31.6	3	22.63	22.06	37.6	2	25.90	26.16
31.7	2	21.55	22.13	37.8	1	25.90	26.29
31.9	3	22.33	22.27	37.9	2	27.00	26.36
32.0	6	22.42	22.34	38.0	1	25.90	26.43
32.1	4	22.58	22.40	38.1	1	25.70	26.50
32.3	3	22.77	22.54	38.3	1	25.80	26.64
32.4	1	22.10	22.60	38.5	1	26.70	26.77
32.5	2	23.65	22.67	38.6	1	25.90	26.84
32.7	4	23.00	22.81	39.0	1	27.10	27.11
32.8	3	23.20	22.88	39.2	2	28.25	27.25
32.9	3	23.27	22.95	39.3	1	26.90	27.32
33.0	1	23.60	23.02	39.6	1	27.00	27.52
33.1	2	23.20	23.08	40.3	1	27.30	28.00
33.2	1	23.90	23.15	40.4	1	27.60	28.06
33.3	3	23.83	23.22	—	—	—	—

them are given together. In the calculation of the ratios, for convenience, the value of *k* from the Sendai specimens was taken as the unit.

TABLE 14.

Viviparus (Sendai, Miyagi-Ken)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
11.0	1	7.90	7.45	20.5	2	14.60	14.07
12.5	1	8.70	8.49	20.6	3	14.30	14.14
12.7	1	8.40	8.63	20.7	1	14.30	14.21
12.8	1	9.00	8.70	20.9	2	14.20	14.35
13.0	2	9.00	8.84	21.0	2	14.40	14.42
13.2	1	8.90	8.98	21.2	1	14.50	14.56
13.5	3	9.23	9.19	21.3	3	14.47	14.63
13.6	1	9.50	9.26	21.4	1	14.90	14.70
13.8	1	9.10	9.39	21.6	1	14.30	14.84
14.5	1	9.50	9.88	21.7	1	14.20	14.91
14.8	2	10.30	10.09	21.8	3	14.73	14.98
14.9	1	9.10	10.16	22,0	2	14.90	15.12
15.0	1	10.50	10.23	22.1	1	14.80	15.19
15.2	1	10.00	10.37	22.3	1	14.90	15.33
15.3	2	10.80	10.44	22.4	1	15.00	15.40
15.4	1	10.00	10.51	22.5	7	15.37	15.47
15.6	1	11.20	10.65	22.6	2	15.30	15.54
15.8	1	10.50	10.79	22.7	1	15.90	15.61
16.1	1	11.60	10.99	22.8	1	15.05	15.68
16.2	1	11.25	11.07	22.9	4	15.80	15.75
16.6	2	11.17	11.34	23.0	7	15.44	15.82
16.7	1	12.00	11.41	23.1	1	15.60	15.89
16.9	1	11.10	11.55	23.2	4	15.83	15.96
17.2	3	11.78	11.76	23.3	3	15.63	16.03
17.3	1	11.60	11.83	23.4	4	15.76	16.10
17.5	2	11.85	11.97	23.6	2	16.00	16.24
17.6	1	11.50	12.04	23.7	1	16.10	16.31
17.7	3	12.42	12.11	23.8	3	15.87	16.38
17.8	2	12.30	12.18	23.9	3	15.97	16.45
17.9	2	12.60	12.25	24.0	3	16.80	16.52
18.0	4	12.38	12.32	24.1	2	16.30	16.59
18.1	2	13.10	12.39	24.2	4	16.58	16.66
18.2	1	12.90	12.46	24.3	4	16.30	16.73
18.3	2	12.30	12.53	24.4	1	16.10	16.80
18.4	2	12.95	12.60	24.5	3	16.50	16.87
18.5	4	12.56	12.67	24.6	1	16.80	16.94
18.6	1	12.40	12.74	24.7	1	16.80	17.01
18.7	1	13.70	12.81	24.8	1	17.10	17.08
18.8	4	13.05	12.88	24.9	5	17.10	17.15
18.9	4	13.95	12.95	25.0	1	17.00	17.22
19.0	1	12.68	13.02	25.1	6	17.98	17.29
19.1	3	13.13	13.09	25.2	1	17.00	17.36
19.3	2	14.20	13.23	25.3	6	17.12	17.43
19.4	2	13.25	13.30	25.4	5	17.13	17.51
19.5	1	13.10	13.37	25.5	4	17.60	17.58
19.6	1	13.70	13.44	25.6	1	18.50	17.65
19.7	1	12.90	13.51	25.7	5	17.90	17.72
20.0	3	13.78	13.72	25.8	3	17.63	17.79
20.3	1	14.70	13.93	25.9	5	17.56	17.86
20.4	2	13.85	14.00	26.0	7	17.44	17.93

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
26.1	1	18.10	18.00	29.3	3	19.70	20.25
26.2	3	17.98	18.07	29.4	2	21.73	20.32
26.3	3	17.37	18.14	29.5	2	19.90	20.39
26.5	1	18.00	18.28	29.6	2	21.13	20.46
26.6	3	18.70	18.35	29.7	2	20.90	20.53
26.7	4	18.70	18.42	29.8	1	20.20	20.60
26.8	3	18.47	18.49	29.9	2	20.50	20.67
26.9	8	18.78	18.56	30.0	2	20.90	20.74
27.0	4	18.78	18.63	30.1	2	20.53	20.81
27.1	4	19.23	18.70	30.3	1	20.60	20.96
27.2	3	19.03	18.77	30.5	1	20.40	21.10
27.3	1	19.30	18.84	30.6	1	20.90	21.17
27.4	2	18.75	18.91	30.7	1	21.00	21.24
27.5	4	19.20	18.98	30.8	1	21.50	21.31
27.6	1	19.35	19.05	30.9	1	21.50	21.38
27.7	2	18.65	19.12	31.0	1	21.40	21.45
27.8	1	19.00	19.19	31.2	3	21.77	21.59
27.9	4	19.63	19.26	31.4	2	21.95	21.73
28.0	3	19.27	19.33	31.8	1	21.70	22.02
28.1	1	19.50	19.41	31.9	1	22.60	22.09
28.2	4	19.38	19.48	32.2	1	22.70	22.30
28.3	2	19.05	19.55	32.4	1	22.80	22.44
28.4	3	19.87	19.62	32.8	1	22.50	22.72
28.5	8	19.48	19.69	34.1	3	24.07	23.64
28.6	8	19.35	19.76	34.3	2	24.40	23.78
28.7	1	20.10	19.83	34.7	1	25.45	24.06
28.8	1	21.40	19.90	36.0	1	25.00	24.99
28.9	2	20.15	19.97	36.2	2	24.65	25.12
29.0	1	20.60	20.04	36.3	1	25.80	25.19
29.1	2	20.05	20.11	36.4	1	25.85	25.27
29.2	4	20.30	20.18	—	—	—	—

TABLE 15.

Viviparus japonicus var. *iwakawai* PILS.

	$x=0.98$		$x=1.00$		$x=1.02$	
	Value of k	Ratio between k	Value of k	Ratio between k	Value of k	Ratio between k
Sendai	0.736	1.000	0.691	1.000	0.645	1.000
Hanamaki	0.748	1.016	0.702	1.016	0.656	1.017

THE GROWTH RELATION BETWEEN THE HEIGHT AND THE WIDTH IN YOUNG SPECIMENS OF *Littorina sitchana* PHIL. AND OF *Sphaerium heterodon* PILS.

(Tables 16 and 17)

It was suspected in the young specimens of *Littorina sitchana* PHIL. from Watanoha that the growth relation between the height and the width might be represented by a straight line, $a=kb$ (Plate 1, thinner two-dotted chain line).

The measurements of 200 young specimens of *Littorina sitchana* PHIL. from Yunoshima and of 103 young specimens of *Sphaerium heterodon* PILS. from Kita-Rokubancho were taken by Miss MATSU FUKUSHIMA and the following equations were obtained:

Height=0.803 x Width for *Littorina sitchana* PHIL. (Table 16),
Height=0.533 x Width for *Sphaerium heterodon* PILS. (Table 17).

From the above relations it appears to me to be true that at least the molluscans which were studied in this paper show a growth relation between the height and the width which may be expressed by $a=kb$ while they are young or during their life.

TABLE 16.

Littorina (Yunoshima, Asamushi)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
3.3	2	2.65	2.65	5.4	10	4.42	4.34
3.5	1	2.80	2.81	5.5	8	4.38	4.42
3.8	2	3.10	3.05	5.6	8	4.54	4.50
4.0	2	3.15	3.21	5.7	15	4.64	4.58
4.1	3	3.33	3.29	5.8	11	4.65	4.66
4.2	7	3.41	3.37	5.9	5	4.80	4.74
4.3	2	3.35	3.45	6.0	14	4.84	4.82
4.4	4	3.48	3.53	6.1	7	4.97	4.90
4.5	4	3.58	3.61	6.2	4	4.95	4.98
4.6	5	3.66	3.69	6.3	4	5.10	5.06
4.7	3	3.73	3.77	6.4	6	5.10	5.14
4.8	6	3.80	3.85	6.5	8	5.20	5.22
4.9	7	3.96	3.93	6.6	5	5.36	5.30
5.0	10	4.06	4.01	6.7	8	5.34	5.38
5.1	7	4.17	4.10	6.8	1	5.20	5.46
5.2	7	4.27	4.18	6.9	4	5.43	5.54
5.3	10	4.31	4.26	—	—	—	—

TABLE 17.

Sphaerium (Kita-Rokubancho, Sendai)

Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.	Width in mm.	No. of shells studied	Average height in mm.	Calculated height in mm.
5.0	8	2.69	2.69	5.5	13	2.95	2.95
5.1	2	2.70	2.74	5.6	12	3.00	3.01
5.2	7	2.79	2.79	5.7	13	3.02	3.06
5.3	6	2.92	2.85	5.8	19	3.08	3.11
5.4	5	2.92	2.90	5.9	18	3.12	3.16

SUMMARY AND CONCLUSIONS.

1. The growth relation between the height and the width of *Littorina*, *Limnaea*, *Viviparus*, and *Sphaerium* can be expressed by the equation $a=kb^x$, in which a and b are the measurements, k the local constant, and x the specific exponent.

2. The value of the specific exponent is constant in a definite species, while the value of the local constant differs according to individuals as well as to locality.

3. In *Limnaea japonica* JAY and *Viviparus japonicus* var. *iwakawai* PILS. $\text{Height}=k\times\text{Width}^{1.00}$.

4. In specimens of *Littorina sitchana* PHIL. of width narrower than 5-7 mm. $\text{Height}=k\times\text{Width}^{1.00}$,
but in specimens of width wider than 5-7 mm.
$\text{Height}=k\times\text{Width}^{0.74}$.

5. In specimens of *Sphaerium heterodon* PILS. of width narrower than 6 mm. $\text{Height}=k\times\text{Width}^{1.00}$,
but in specimens of width wider than 6 mm.
$\text{Height}=k\times\text{Width}^{1.26}$.

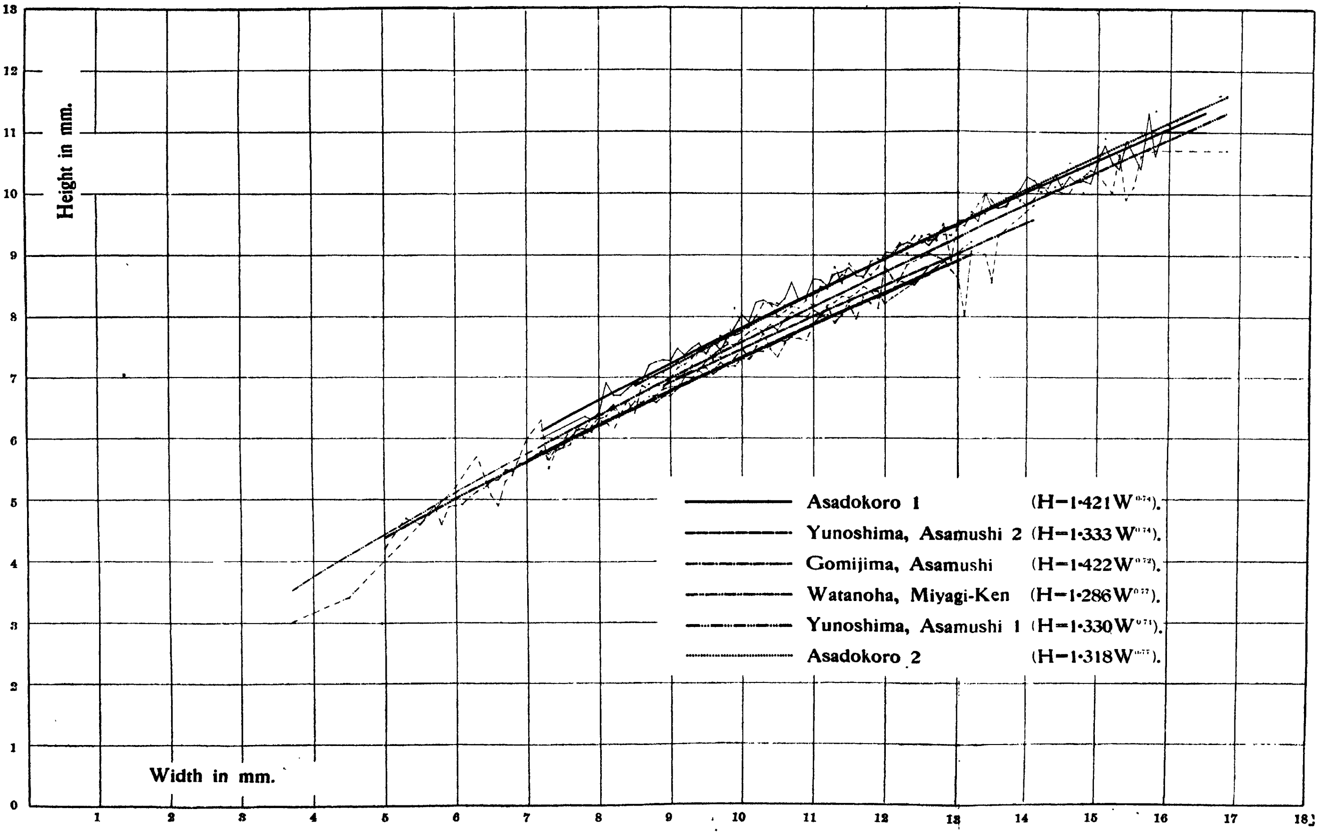
6. We can determine a probable value of the specific exponent from a number of specimens smaller than 500.

7. When a probable value of the specific exponent is determined, the ratio between the corresponding values of the local constant shows a comparative degree of individual or of mean local variation.

March 30, 1926.

PLATE 1.

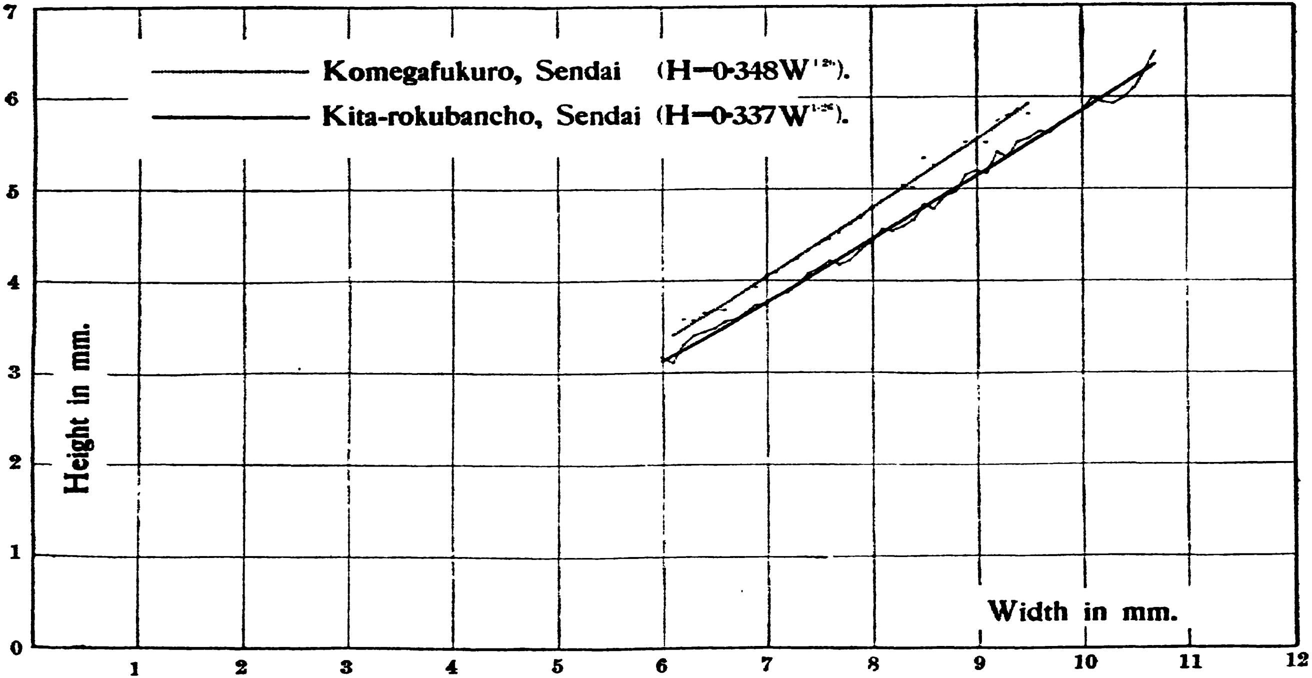
E. NOMURA. *Littorina sitchana* PHIL.



The plate was plotted from Tables 1–6. H height, W width. The thinner lines denote the relations between the height and the width obtained from the actual measurements, and the thicker lines denote the relations expressed by the equations.

PLATE 2.

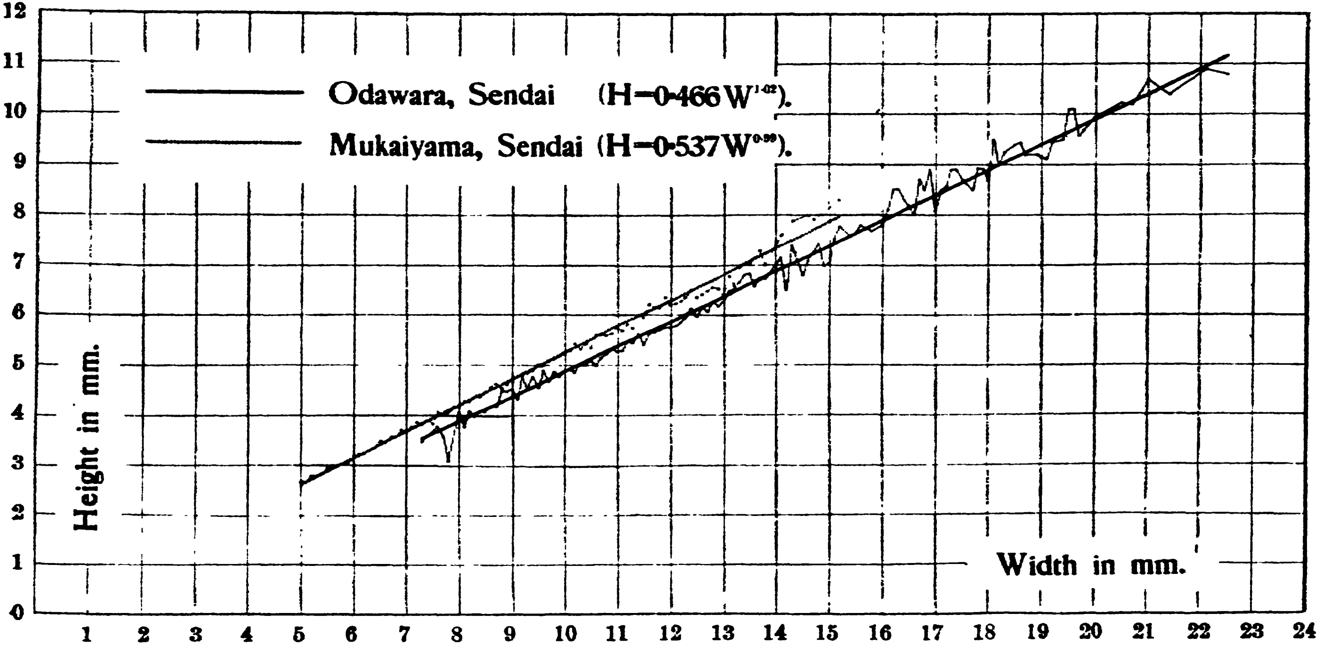
E. NOMURA. *Sphaerium heterodon* PILS.



The plate was plotted from Tables 8 and 9. H height, W width. The thinner lines denote the relations between the height and the width obtained from the actual measurements and the thicker lines denote the relations expressed by the equations.

PLATE 3.

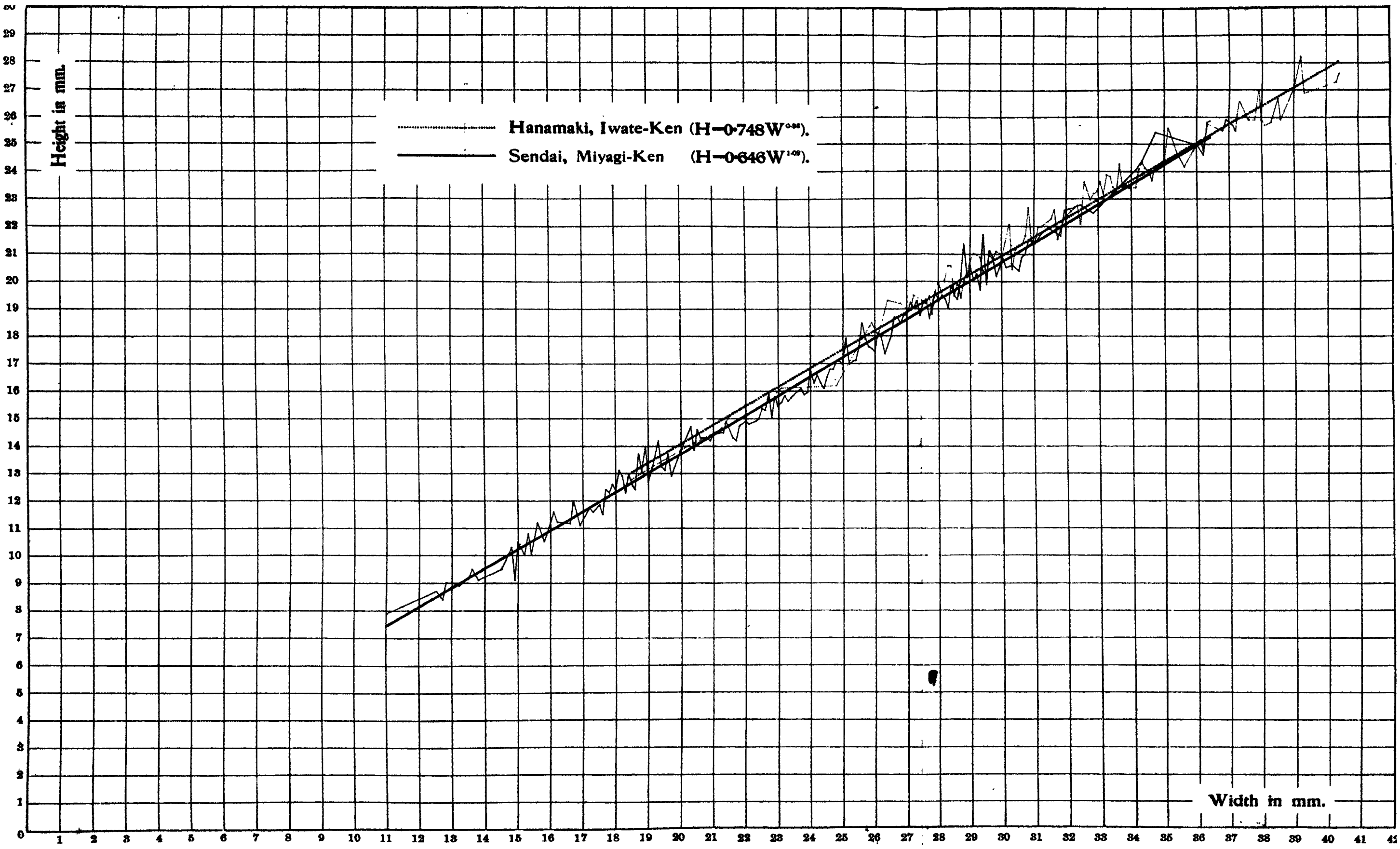
E. NOMURA. *Limnaea japonica* JAY.



The plate was plotted from Tables 10 and 11. H height, W width. The thinner lines denote the relations between the height and the width obtained from the actual measurements, and the thicker lines denote the relations expressed by the equations.

PLATE 4.

E. NOMURA. *Viviparus japonicus* var. *iwakawai* PILS.



The plate was plotted from Tables 13 and 14. H height, W width. The thinner lines denote the relations between the height and the width obtained from the actual measurements, and the thicker lines denote the relations expressed by the equations.

Some preliminary Notes on the Behavior of the Holothurian, *Caudina chilensis* (J. MÜLLER).*

BY

TOSHIHIKO YAMANOUCHI.

(Zoological Laboratory, College of Science, Kyoto Imperial University.)

The present investigations were undertaken at the suggestion of Prof. HATAI and were carried on along the coast near the Asamushi Marine Biological Station during the thirty days from the middle of August to the middle of September 1925.

HABITAT AND GENERAL BEHAVIOR OF THE ANIMAL.

This animal lives in fine sand rich in organic substances and is found most abundantly at a shallow sea bottom where the depth of the water is about 30 cm. at low tide.

For the convenience of explaining the posture of this animal in the sand, let us devide its body into two portions, trunk and tail. The trunk is the anterior portion, which is specially swollen, in distinction to the tail or posterior portion, which is slender and contains cloacal muscles. The whole body of the animal always lies embedded in sand with the posterior end directed upward and the anterior end downward, and in most cases the posterior portion of the tail lies perpendicular to the surface of the sea bottom, while the trunk lies obliquely, making an inclination of about 30° to the surface. In general, when the animal is in the natural position the end of the tail measures about 1.5 times the length of the trunk (Fig. 1).

Generally, the largest specimens are found nearest the coast. This fact suggests that the animal migrates toward the coast with the growth of the body. I made an experiment on some individuals in connection with this behavior, but I was unable to recognize such movements in spite of a continuous observation for four days.

Wherever the animal lives we find, without fail, an excremental

---

* A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

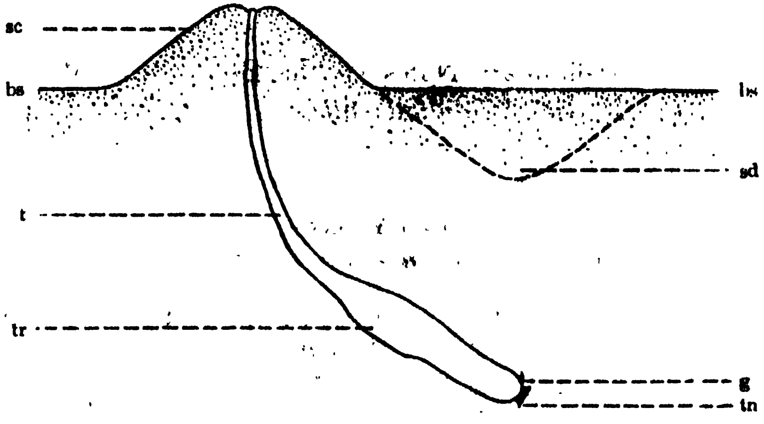


Fig. 1. Diagrammatic vertical section of a habitat to show the natural posture of *Caudina*. bs–bs, surface of sea bottom; g, genital papilla; sc, sand cone; sd, sand depression; t, tail; tn, tentacles; tr, trunk.

pile which is mainly composed of sand particles, generally in the form of a cone. Let us call it hereafter the "sand cone" (Fig. 1. sc). The base of a sand cone generally makes a circular, sometimes an elliptical or oval, outline and its diameter sometimes reaches 30 cm.

We often find a depression on one side of a sand cone (Fig. 1, broken line). In this case the anterior end of the animal is always below the center of the depression. From this we may infer that the animal makes a depression because it swallows sand, and in turn, makes a sand cone because it excretes sand previously swallowed.

When the shallow sea where the animals live is violently disturbed, for instance, by a strong storm, most of the sand cones break down and disappear.

In collecting the animals, we utilize their natural posture in the sand, for at first we catch the tail portion at the axis of a sand cone with one hand and then begin to dig with the other hand in search of the trunk. In the presence of a sand depression, knowing the position of the anterior end, we can at once dig out the whole body of the animal with both hands.

It is worthy of note that when we pull the tail of the animal, a constriction, caused by the contraction of cloacal muscle, makes an accurate distinction between the tail and the trunk.

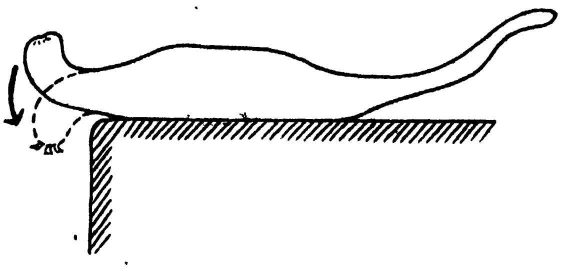


Fig. 2. *Caudina* placed on a PETRI dish (full line) showing positive geotropism (broken line).

I put a PETRI dish upside down in sea water and then placed an individual on this dish, the anterior portion of its trunk being projected over the edge of the dish (Fig. 2.). In this experiment I noticed that the anterior portion of the trunk curved downward, showing positive geotropism; that the oral tentacles, following the contraction of the oral muscles, came out of the mouth cavity; and that the rhythmic contraction of the circular muscle of the body propagated postero-anteriorly. This rhythmic contraction occurs at intervals from 34 sec. to 45 sec. (mean interval 37 sec.).

I placed two individuals directly on sand in sea water. When they fully showed positive geotropism, I reversed their bodies, making the curved anterior ends toward upward. At intervals from 21 sec. to 207 sec. they showed positive geotropism by turning the anterior ends vertically. From this we may infer that in the geotropism of the anterior portion of the animal the bending gives rise along the vertical plane which contains the body axis and any line along the body surface where attaches the substratum.

The anterior end of the body curves downward, the tentacles reach the surface of the sand, and then the animal, following the rhythmic contraction of the circular muscle of the body which propagates postero-anteriorly, begins to force itself gradually into the sand. During this process, I have noticed that the contraction of the circular muscle at the anterior end results in a somewhat sudden thrust, but the relaxation brings about a slight retraction, followed by a shortening of the anterior portion of the body, which is caused by the contraction of the longitudinal muscles.

In general, the duration of time from the beginning to the completion of burial is proportional to the size of the body.

When the animal lies in the sand, the genital papilla is always found on the upper surface of the body. In order to ascertain whether the posture just described is normal or not, I have carried on some experiments.

1) After the complete intrusion of 20 individuals into the sand, I have tested their newly occupied postures and enumerated:

in 17 individuals, genital papillae were found on the upper surface of the body,
in 1 individual, genital papilla was found on the oblique upper surface of the body,
and in 2 individuals, genital papillae were found on the lateral surface of the body.

2) After the complete intrusion into the sand of 21 individuals, whose genital papillae were situated initially on the upper surface of the body, I have enumerated:

in 18 individuals, genital papillae were found on the upper surface of the body,
in 1 individual, genital papilla was found on the oblique lower surface of the body,
and in 2 individuals, genital papillae were found on the lower surface of the body.

3) After the complete intrusion into the sand of 11 individuals, whose genital papillae were situated initially on the lateral surface of the body, I have enumerated:

in 8 individuals, genital papillae were found on the upper surface of the body,
in 1 individual, genital papilla was found on the oblique upper surface of the body,
and in 2 individuals, genital papillae were found on the lateral surface of the body.

4) When the animal, whose genital papilla was situated initially on the lower surface of the body, was placed directly on sand, it began to force itself into the sand with the genital papilla at the given position, but when the trunk had been buried nearly half its length, the animal turned the posterior portion of the body, as is shown in Fig. 3, making the genital papilla occupy the upper surface of the body.

When 44 individuals, whose genital papillae were situated initially on the lower surface of the body, showed several degrees of intrusion, I enumerated the number of individuals which showed a similar position of the genital papillae, and the results are given in Table 1. From this table we may infer that in most cases when nearly half the length of the trunk is buried in sand the animals make their bodies occupy

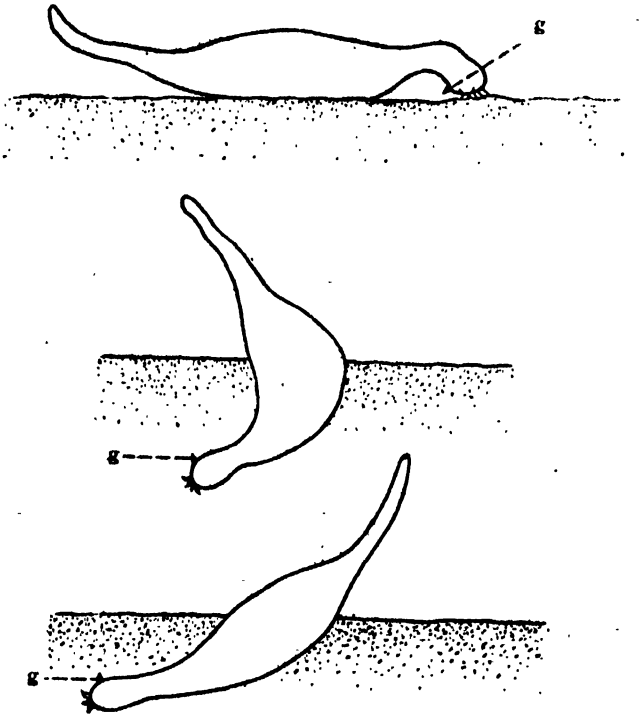


Fig. 3. The intrusion into the sand of *Caudina*, whose genital papilla was situated initially on the lower surface of the body. g, genital papilla.

TABLE 1.

Situation of genital papillae	Intrusion of whole length of trunk	Intrusion of 2/3 length of trunk	Intrusion of 1/2 length of trunk	Intrusion of 1/3 length of trunk	Intrusion of 1/4 length of trunk
Upper surface	19	6	8	1	
Oblique Upper surface	2		3		
Lateral surface				1	1
Oblique Lower surface				1	
Lower surface			1		1

a position which brings the genital papillae on the upper surface of the body.

In summarizing the results from the above experiments, it seems to be true that in the normal posture of the animal the genital papilla is situated on the upper surface of the body.

When the posterior half of the animal was embedded in the sand and the remaining anterior half was erected vertically, the anterior end then curved downward, showing positive geotropism. Within the limit of my observation, however, the animal came out once more from the sand and then made a definite intrusion in a similar manner to that stated in the above.

When a large animal was kept in a laboratory aquarium in which the sea water was constantly renewed, it showed no changes from its normal behavior on the first day. On the second day, however, its tail portion came out of the sand and swang to and fro, and on the third or fourth day the greater part of the body or the whole body came out of the sand and the animal moved about at random in the aquarium, its nature of intruding into the sand having been lost. In general, small-sized specimens continued the normal posture in the sand for many days and I could observe some specimens which kept the normal posture from 10 to 14 days.

SAND CONE.

Sand cones were broken down by a severe storm of August 28. The sea became almost calm at 1 A. M. August 29. At 3 P. M. of the same day I found sand cones which were newly formed by the animals. I measured the basal diameters of 36 sand cones and obtained 9 cm. as the average value.

In the actual determination of the weight of dried sand from a sand cone, the basal diameter of which was 9 cm., I obtained 92 gms. as the probable weight of the sand which was contained in the sand cone. Since 9 cm. is the average basal diameter of the sand cones which were newly formed by the animals during the interval from 1 A. M. to 3 P. M. or 14 hours, we may infer, taking 92 gms. per 14 hrs. as the basis of the calculation, that the animal swallows and excretes sand at the average of

6.57 gms. per hour,
158.00 gms. per day,
and 57670.00 gms. per year.

Twenty hours after the beginning of the reformation, the sand cones showed somewhat irregular shapes and became low in comparison with the corresponding diameter.

Before leaving the subject, I desire to express my sincere thanks to Prof. Hatai for his kind guidance and encouragement, and to Assist. Prof. Kokubo, Mr. Ogawa and Mr. Kawamoto for their assistance and warm friendship during my stay at Asamushi. I am also indebted to Prof. E. Nomura for his kindness of reading my manuscript and giving me valuable criticisms.

Embryologische Studien an *Heloniopsis breviscapa*.

VON

TOMOWO ONO.

1. EINLEITUNG.

Vom embryologischen Standpunkt aus bilden Liliaceen eine sehr interessante Familie; viele bedeutende Untersuchungen wurden schon bei dieser Familie von den verschiedenen Forschern gemacht. *Heloniopsis*, auch eine Gattung dieser Familie, kommt aber ausschliesslich in Ostasien vor und hat bis jetzt fast gar keine Bearbeitung in dieser Richtung erfahren. Eine Spezies dieser Gattung, *H. breviscapa*, wächst allgemein in der Nähe von Sendai. Embryologische Eizelheiten dieser Pflanze werden unten beschreiben werden.

Als Fixierungsmethoden der Fruchtknoten benutzte ich die folgenden: 1) Die 24 Stunden Fixierung mit FLEMMINGS Lösung nach 1 oder 2 Minuten Behandlung mit CARNOYS Lösung, 2) 24 Stunden Fixierung nur mit FLEMMINGS Lösung, 3) 18 Stunden Fixierung mit BOUINS Flüssigkeit. Jede von diesen drei zeigte gleich ein sehr gutes Resultat. Es war aber für die karyologische Untersuchung die letztgenannte Methode nicht geeignet.

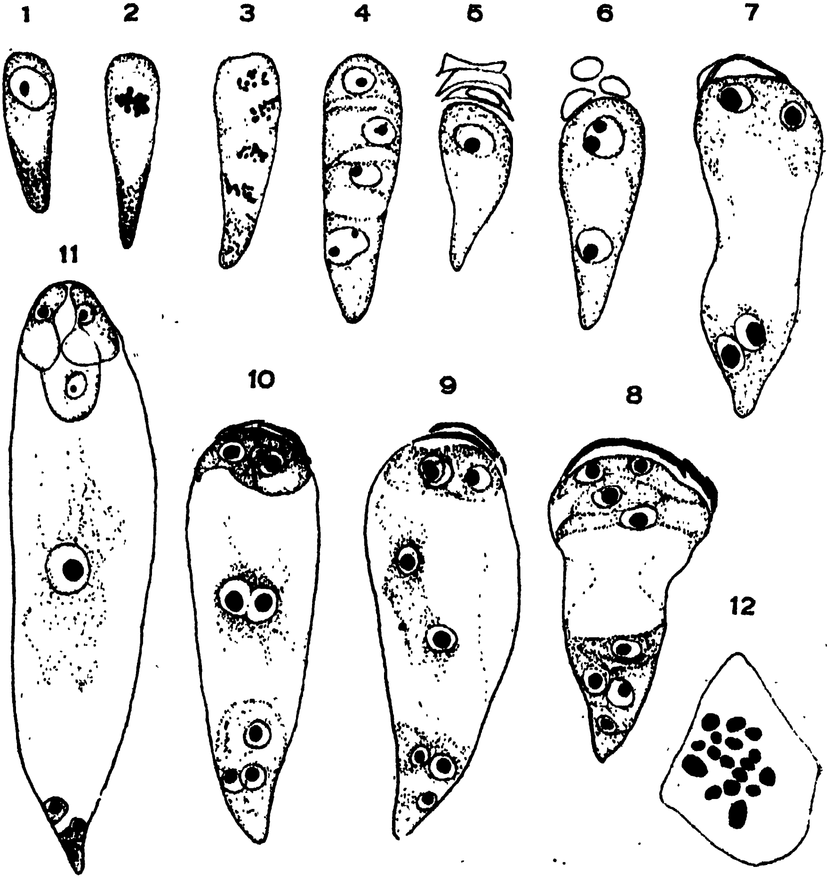
Nach der Einbettung in Paraffin wurden die Materialien in 10 zu 20 μ Dicke geschnitten. Zur Färbung habe ich HEIDENHAINS Eisenhämatoxylin oder Safranin-Lichtgrün benutzt. Die letztgenannte Methode ergab vortreffliche Ergebnisse bei der Zellwandfärbung.

Diese Untersuchung wurde im biologischen Institut der kaiserlichen Universität zu Sendai unter der Leitung von Prof. Dr. M. TAHARA ausgeführt. Ich möchte hier für seine allseitige Unterstützung und Anregung meinen herzlichsten Dank auszusprechen.

2. EMBRYOSACKBILDUNG.

Die Embryosackbildung bei *Heloniopsis breviscapa* verläuft ganz normal. Verschiedene Anomalien der Embryosackbildung wurden schon häufig bei Liliaceen gefunden; normale Entwicklung ist bei dieser Familie ziemlich selten. Solche sind bis jetzt bei nur einigen Gattungen geschildert, nähmlich bei *Tricyrtis* (IKEDA, 1902), *Gloriosa* (AFZELIUS, 1918) und *Colchicum*[1] (HEIMANN-WINÁWER, 1919). Es würde merkwürdig sein, dass diese Gattungen und *Heloniopsis* alle den Melanthioideen, der primitivsten Unterfamilie der Liliaceen, angehören.

Die Embryosackmutterzelle liegt im Zentrum des Nucellarhöckers und wird als ausdehnende und grosskernhaltige Zelle charakterisiert.



Textfig. I. 1-11, Embryosackentwicklung (×680), 12, Heterotypische Metaphase in der Embryosackmutterzelle (×700).

---

[1] Zitiert nach SCHÜRHOFF (1925).

Textfig. I, 1–4 zeigen die Tetradenbildung der Embryosackmutterzelle. Die erste Kernteilung der Embryosackmutterzelle ist heterotypisch. In der Metaphase dieser Teilung treten 17 Gemini[1] auf (Textfig. I, 12). Dann folgt die zweite, homöotypische Kernteilung. Durch diese Teilungen entstehen vier Megasporen, welche sich in einer Reihe anordnen (Textfig. I, 4).

Die unterste Zelle dieser so gebildeten vier Megasporen wird die definitive primäre Embryosackzelle, nimmt an Grösse bedeutend zu und somit verdrängt die drei übrigen Megasporen (Textfig. I, 5).

Textfig. I, 6 zeigt das zweikernige Stadium des Embryosackes. Die beiden neugebildeten Kerne wandern auseinander nach beiden Polen hin, während das Plasma immer mehr vacuolisiert wird, und zuletzt entsteht im Zentrum eine grosse Vacuole (Textfig. I, 7). Bald darauf sieht man die zweimaligen sukzessiven Kernteilungen, wodurch ein vier-, zuletzt achtkerniger Embryosack entsteht (Textfig. I, 7, 8). Die beiden oberen von den vier Kernen im Mikropylarende bilden sich zu den zwei Synergiden aus und die beiden unteren zu einem Eikern und einem Polkern. Ebensoauch entstehen im Antipodalende die Antipoden und ein Polkern (Textfig. I, 8, 9). Die beiden Polkerne kommen einander langsam näher und verschmelzen zuletzt miteinander in der Mitte des Embryosackes (Textfig. I, 9, 10). In dem durch die Verschmelzung der beiden Polkerne entstandenen Kern sind anfangs zwei Nukleolen zu erblicken, die jedoch bald zu einem grossen Nukleolus verschmelzen. Der Embryosack im fertigen Zustand ist gross und plasmaarm (Textfig. I, 11). Im Verhältnis dazu ist die Eizelle ziemlich klein. Sie ist ellipsoidisch; im gespitzten Teile dieser Zelle liegt der Kern. Die beiden Synergiden haben je eine grosse Vacuole am unteren Ende, und der Kern liegt am oberen Ende. Antipodalzellen sind zuerst einkernig, werden aber später zweikernig.

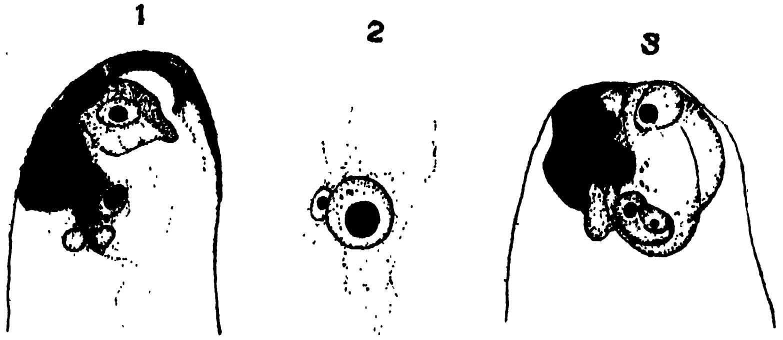
3. BEFRUCHTUNG.

Die Vereinigung der beiden Polkerne wird vor der Befruchtung ausgeführt. So sieht man im Zentrum des fertigen Embryosackes einen grossen sekundären Endospermkern mit einem grossen Kernkörperchen.

---

[1] MIYAJI hat schon in den Wurzelspitzen 34 Chromosomenzahlen festgestellt (ISHIKAWA, 1916).

Der durch die Mikropylare zum Nucellusscheitel wachsende Pollenschlauch dringt dann durch eine der Synergiden in die Eizelle vor. Das Plasma des Schlauches ist sehr dicht und färbt sich so intensitiv, dass die Beobachtung der Kerne im Pollenschlauche erschwert wird. Zur Zeit der Befruchtung ist eine intensitiv gefärbte halbmondförmige Masse in der Nähe der Eizelle sichtbar. Diese ist nichts anders als eine der zerstörten Synergiden, in welche ein Pollenschlauch seinen Inhalt entleert hat.

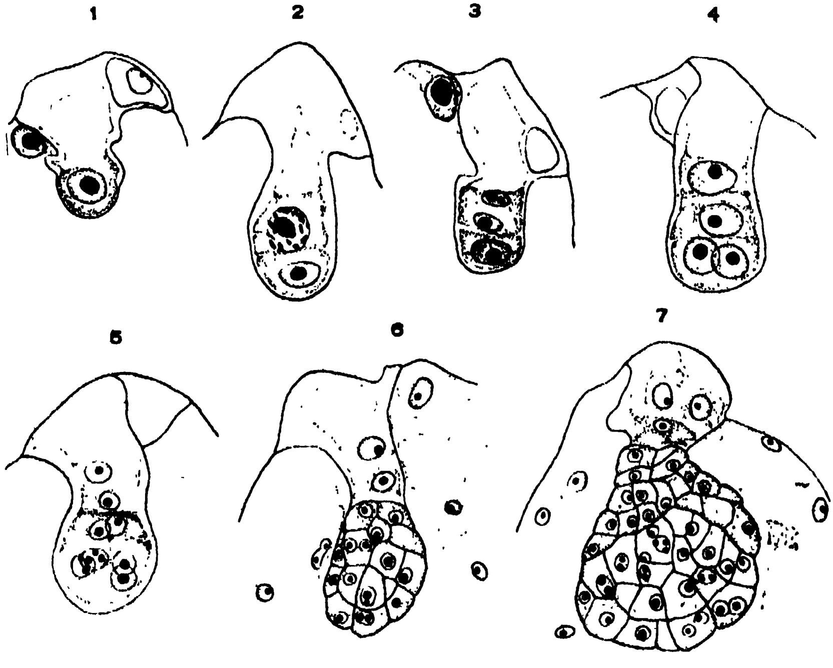


Textfig. II. Doppelbefruchtungsverlauf (× 1000).

Textfig. II, 1–3 zeigen die Verschmelzungsvorgänge der männlichen Kerne mit dem Eikerne und dem sekundären Embryosackkerne. Bei diesen Stadien nimmt der Embryosack an Grösse bedeutend zu, und ist ziemlich schwer, den ganzen Embryosack in einem Schnitte zu erblicken. Die aus dem Pollenschlauche ausgetretenen zwei kugeligen kleinen Spermakerne (Textfig. II, 1) sind beinahe gleichgestaltet. In jedem fehlt vorerst ein Nukleolus, wie bei der naheverwandten Gattung, *Tricyrtis* (IKEDA, 1902). Die beiden Spermakerne liegen zuerst nebeneinander (Textfig. II, 1). Dann dringt der eine in die Eizelle ein (Textfig. II, 3), und der andere wandert gleichzeitig zum sekundären Embryosackkerne (Textfig. II, 2). Zu diesem Zeitpunkt tritt ein Nukleolus in diesem Spermakerne sehr deutlich auf. Im befruchteten Eikerne sieht man öfters zwei Nukleolen. Und im sekundären Embryosackkerne, welcher schon mit einem Spermakerne vereinigt ist, sind zuerst zwei ungleich grosse Nukleolen zu sehen, der kleinere stammt vom Spermakerne ab Die befruchtete Eizelle bekleidet sich mit einer Zellulosemembran und geht später zu der Ausbildung des Embryos über.

4. Embryoentwicklung.

Der einzellige Proembryo ist vorerst flaschenförmig, mit einer grossen Vacuole im basalen Teile (Textfig. III, 1). Die erste Teilungswand wird quer angelegt und trennt eine kleine Zelle vom aufgeblasenen Fussteile. Die zweite Kernteilung kommt immer in der Basalzelle des Proembryos vor. Textfig. III, 2 zeigt die Prophase dieser Teilung. Nach diesen Vorgängen entsteht ein dreizelliger Proembryo; die drei Zellen liegen immer in einer Reihe (Textfig. III, 3). Die Spitzenzelle eines solchen Proembryos ist das ursprüngliche Kotyledon und teilt sich



Textfig. III. Embryoentwicklungsverlauf. (× 1320).

etwas früher als die zwei übrigen Zellen (Taxtfig. III, 4). Textfig. III, 5 zeigt die Weiterentwicklung des Embryos; in dessen Spitzenzelle finden wir schon vier Kerne. Die zwei obersten Zellen sind etwas plasmaarm und bleiben als Suspensorzellen bestehen, welche weiteren Zellteilungen nicht unterworfen sind. Zuletzt wird ein massiver Embryo ausgebildet (Textfig. III, 6, 7).

In Bezug auf die Embryoentwicklung gibt es vier Typen bei den Monokotyledonen d. i. Alisma-, Pistia-, Lilium-, und Orchid-Typus (COULTER und CHAMBERLAIN, 1903). Bei Liliaceen finden wir meistenfalls den Lilium-Typus z. B. *Lilium* (COULTER, 1897), *Erythronium* (SCHAFFNER, 1901), *Tulipa* (ERNST, 1901), *Asparagus* (ROBBINS und BORTHWICK, 1925) u. a. Bei diesem Typus finden die Teilungen nach der ersten Querzellwandbildung unregelmässig statt. Wie oben erwähnt, schreitet die Embryoentwicklung bei *Heloniopsis*, wenigstens in den frühen Stadien, sehr regelmässig vor und liefert eine Ausnahme in den Liliaceen. Dem Lilium-Typus gehört sie also nicht an, sondern vielmehr dem Alisma-Typus.

5. ENDOSPERMBILDUNG.

Betreffs der Endospermbildung der Angiospermen gibt es zwei Haupttypen, nämlich die nukleare und die zellulare. Die nukleare Endospermbildung ist dadurch charakterisiert, dass sie mit einer raschen Kernvermehrung einsetzt, auf die erst später die Zellbildung mit unbestimmter Zellwandorientierung folgt. Im Gegensatz hierzu ist die zellulare durch sofortige Zellbildung mit bestimmter Wandorientierung schon nach der ersten Kernteilung charakterisiert. Ausser diesen zwei Haupttypen gibt es noch einen anderen Typus, d. i. den Helobiae-Typus, ein Merkmal von der Reihe Helobiae. In diesem Typus, unmittelbar nach der Teilung des primären Endospermkerns, entstehen zwei ungleich grosse Zellen; die kleinere sitzt über den Antipoden und die grössere nimmt den übrigen Teil des Embryosackes ein. Die freien Kernteilungen gehen wiederum in diesen zwei Zellen vor. Aber in der kleineren bleiben die Kernteilungen auch bisweilen aus. Der antipodale Abschnitt des Endosperms von diesem Typus ist von einigen Autoren Basalzelle oder Basalapparatus genannt worden. Der Helobiae-Typus kommt nach den neueren Untersuchungen nicht nur in der Reihe der Helobiae, sondern auch in vielen Familien von Mono- und Dikotyledonen vor (SAMUELSSON, 1913, PALM, 1915, SCHÜRHOFF, 1924 u. a.).

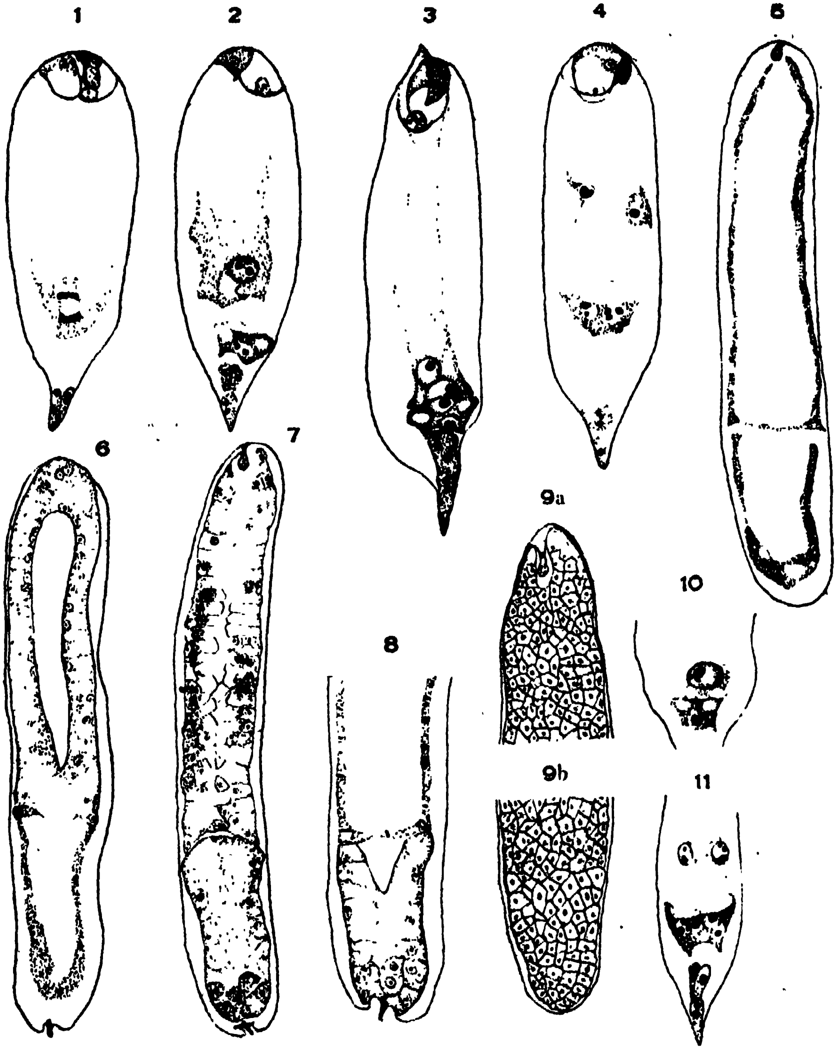
Es ist bemerkenswert, dass sich die verschiedenen Typen der Endospermbildung manchmal in ein und derselben Familie befinden. Bei Liliaceen waren bis vor kurzem nur die nuklearen bekannt worden, aber im Jahre 1924 teilte Dahlgren in seiner Abhandlung Seeliebs[1] Entdeckung von der Helobiae-Typus-Endospermbildung bei *Tofieldia calyculata* mit. Es sagt: „Nach der ersten Teilung des primären Endospermkerns, wird der Embryosack in eine grosse mikropylare und eine kleine basale Zelle zerlegt. Die weitere Entwicklung erfolgt durch freie Kernteilung und später nachfolgende Zellbildung sowohl in der basalen wie auch in der mikropylaren Zelle, nur dass die Teilungen in der ersteren viel langsamer erfolgen als in der grösseren Tochterzelle."

Die Endospermbildung bei *Heloniopsis* verläuft ähnlich wie diese. Nach der Doppelbefruchtung findet man einen grossen, kugeligen, primären Endospermkern mit zwei verschieden grossen Nukleolen im Zentrum oder etwas näher beim Antipodalende des vergrösserten Embryosackes. Die erste Teilungsspindel des Endospermkerns orientiert sich in der Längsachse des Embryosackes (Textfig. IV, 1). Zwischen den durch diese Teilung entstandenen beiden Tochterkernen tritt keine feste Zellwand, sondern nur eine Hautschicht auf. Dies ist auch der Fall bei *Ottelia, Hypericum* (Palm, 1915, 1922) und *Aponogeton* (Afzelius, 1920). Textfig. IV, 2 zeigt die so abgegrenzten zwei Kammern des Endosperms; der obere mikropylare Teil ist plasmaarm und hat im Innern einen grossen kugeligen Kern mit drei Nukleolen. Der untere antipodale Teil hat dagegen eine ▼-förmige dichtere Plasmamasse mit zwei Vacuolen und einem Kern. Da die untere Kammer auf dem Antipodalende liegt, scheint es, als ob sie von den Antipoden abstammte. Die aufeinanderfolgenden Stadien der Entwicklung dieser Kammer sind in Textfig. IV, 10, 11 abgebildet.

Die frühen Kernteilungen in diesen zwei Kammern finden sich meistens gleichzeitig (Textfig. IV, 3, 4), aber auch manchmal etwas früher in der oberen Kammer als in der unteren. Durch die wiederholten Mitosen entstehen schliesslich viele freie Kerne in den beiden Kammern. Diese Kerne verteilen sich gleichmässig im plasmatischen Wandbelag (Textfig. IV, 5). Die Teilungen in diesem Wandbelag

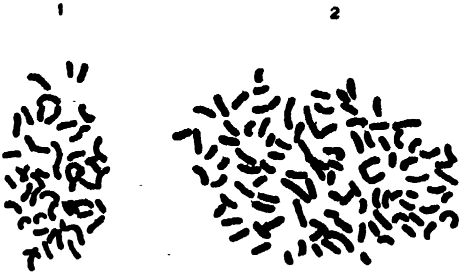
---

[1] Seeliebs Mitteilung: „Beitrag zur Entwicklungsgeschichte von *Tofieldia calyculata* (L) Wahlenb." (Bot. Notiser 1924) war mir leider unzugänglich und kann nicht hier eingehend zitiert werden.



Textfig. IV. Endospermbildungsverlauf. 1-4, 10-11 (×240), 5-9 (×60).

schreiten von oben nach unten vor, so dass man in demselben Präparat alle Teilungszustände vereinigt finden kann. In der Metaphase, wie Textfig. V, 1 zeigt, treten vor uns 51 triploide Chromosomen auf. Der protoplasmatische Wandbelag ist zuerst äusserst dünn, verdickt sich aber bei weiterer Entwicklung mehr und mehr. Vor der Zellwandbildung im Endosperm kommt selten Kernverschmelzung an der antipodalen Kammer vor. Textfig. V, 2 zeigt vielleicht einen solchen



Textfig. V. 1, Mitosis des Endospermkerns. 2, Mitosis des verschmolzenen Kerns im Endosperm (×750).

Kern im Teilungsstadium. Dort sieht man etwa 102 Chromosomen. Wie Textfig. IV, 6 zeigt, wird die Zellwandbildung etwas früher in der oberen Kammer ausgeführt als in der unteren. Dieser Vorgang schreitet auch von oben nach unten vor. Wenn man die mit 10% Kalilauge behandelten jungen Samen unter das Mikroskop bringt, sieht man klar, dass die mikropylare Kammer mit gelber Masse gefüllt, die untere dagegen ganz durchsichtig ist. Diese Tatsache erweist, dass die obere Kammer schon Zellwände gebildet hat, jedoch die untere nicht. Dieser Entwicklungsmodus des Endosperms geht aber manchmal in entgegengesetzter Richtung vor, d. i. die Zellwandbildung findet vorerst manchmal in der unteren Kammer statt[1] (Textfig. IV, 8).

Der durch die Wandbildung entstehende Zellkomplex ist zuerst einschichtig in den beiden Kammern und wird erst später mehrschichtig (Textfig. IV, 7). Textfig. IV, 9 zeigt ein beinahe fertiges Stadium der Endospermentwicklung. Im fertigen Endosperm lässt sich die Grenze zwischen den beiden Kammern nur sehr schwer feststellen. Manchmal scheint das Endosperm, wie Textfig. IV, 9 zeigt, als einheitliche Plasmamasse. Aber bei näherer Beobachtung kann man eine winzige Einschnürung im unteren Teile des Endosperms finden. Sie bedeutet die Grenze zwischen den beiden, oberen und unteren, Abschnitten des Endosperms.

---

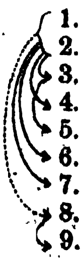
[1] Unter den von mir untersuchten 18 Fällen habe ich 3 solche Fälle gefunden.

Die Verschiedenheit der Endospermbildung der Angiospermen hat schon die Aufmerksamkeit vieler Forscher auf sich gezogen. Dabei taucht zunächst immer die Frage auf, welche von den Endospermbildungen phylogenetisch die ursprünglichere sei.

SAMUELSSON (1913) hat in seinem *Bicornes*-Werke beschrieben, dass das zellulare Endosperm phylogenetisch jünger sei, als das nukleare. Im letzten Jahre ist DAHLGREN (1924) bei der Untersuchung über die Endospermbildung bei Kompositen auch auf diese Frage eingegangen und meint folgendes: „Ich bin fest überzeugt, dass der nukleare Typus der älteste ist. Das ab initio zellulare Endosperm muss folglich an verschiedenen Stellen im System selbständig entstanden sein." Jedoch hat SCHNARF (1925) in seiner kritischen Untersuchung über den Helobiae-Typus eine entgegengesetzte Ansicht dargelegt, nach der das helobiale Endosperm aus dem zellularen entstanden sei, indem die Wandbildung bis auf die Bildung der Querwand (bzw. Hautschicht) zwischen den zuerst entstehenden zwei Endospermkernen unterdrückt wurde, und die weitere Auswirkung dieser Tedenz zum nuklearen Typus führt.

Es ist eine interessante Tatsache, dass diese verschiedenen Typen oft in derselben Reihe oder Familie nebeneinander vorkommen. Saxifragaceen (GÄUMANN, 1919) sind unter anderen besonders in dieser Hinsicht ausgezeichnet.

Bei den Monokotyledonen kennen wir aber bis jetzt keinen sicheren Fall der zellularen Endospermbildung,[1] und zwar haben die höher entwickelten Familien dieser Abteilung meistens das nukleare Endosperm, wie die folgende Tabelle zeigt:



	Monokotyledonenreihe[2]	Typus des Endosperms[3]
1.	Helobiae	(H, N)
2.	Liliiflorae	(H, N)
3.	Enantioblastae	(H)
4.	Cyperales	(H)
5.	Glumiflorae	(N)
6.	Scitamineae	(N)
7.	Gynandrae	Endosperm fehlt
8.	Spadiciflorae	(H, N)
9.	Pandanales	(N)

---

[1] Bei Policarpicae, eine als Ahn der Monokotyledonen angenommene Dikotyledon-Reihe, werden ausser dem Helobiae- und nuklearen Typus die zellularen Endosperme gesehen.

[2] Phylogenetischer Plan nach R. WETTSTEIN.

[3] H. zeigt helobiales, N nukleares Endosperm.

Diese Tatsache dürfte uns ungezwungen zu dem Schluss leiten, dass das zellulare Endosperm ursprünglicher als die beiden anderen sei, da die Monokotyledonen, wie heute allgemein angenommen wird, aus den Dikotyledonen herstammen sollen.

Bei den Liliaceen finden wir den nuklearen Typus weit verbreitet. Bis vor kurzem hatten wir nur einzige Beispiel der helobialen Endospermbildung gekannt. Das ist *Tofieldia*, und mein diesmalige Untersuchungsobjekt *Heloniopsis* bietet das zweite Beispiel davon dar. Es mag merkwürdig sein, dass diese zwei Gattungen der primitivsten Unterfamilie von Liliaceen, Melanthioideen angehören. Das Vorkommen von Helobiae-Typus-Endospermbildung bei Melanthioideen dürfte nicht nur eine Unterstützung der Annahme sein, dass das helobiale Endosperm ursprünglicher ist als das nukleare, sondern auch der Ansicht SCHNARFS, dass das helobiale Endosperm ein unterdrücktes zellulares Endosperm ist.

6. ZUSAMMENFASSUNG.

1) Viele Anomalien der Embryosackentwicklung sind schon bei den Liliaceen gefunden wurden. Die Embryosackentwicklung bei *Heloniopsis* geht aber ganz normal vor sich; die sog. Doppelbefruchtung kommt auch bei dieser Pflanze vor.

2) Die haploide Chromosomenzahl von *Heloniopsis breviscapa*, bestimmt bei der Heterokynese der Embryosackmutterzelle, beträgt 17. Bei den Mitosen der Endospermbildung treten 51, also triploide Chromosomen auf.

3) Die Embryoentwicklung schreitet in den früheren Stadien sehr regelmässig vor. Dies ist eine Ausnahme von den Liliaceen, weil die meisten Mitglieder dieser Familie nach dem sog. Lilium-Typus ihre Embryoentwicklung ausführen.

4) Die Endospermbildung bei *Heloniopsis breviscapa* ist vom sog. Helobiae-Typus; nach der ersten Teilung des primären Endospermkerns wird der Embryosack in zwei ungleiche Kammern geteilt. Zwischen ihnen bleibt aber eine echte Zellulosewandbildung aus. Die Vielzellbildung kommt später gleich bei diesen zwei Kammern vor.

5) Unter den drei Typen der Endospermbildung scheint der zellulare der ursprünglichste zu sein, und der nukleare dürfte erst später aus dem zellularen oder dem helobialen entspringen.

LITERATURVERZEICHNIS.

1) Afzelius, K. 1918. Zur Entwicklungsgeschichte der Gattung *Gloriosa*. Acta Horti Bergiani, Bd. 6, Nr. 3, S. 3–12.

2) Afzelius, K. 1920. Einige Beobachtung über die Samenentwicklung der Aponogetonaceae. Sv. Bot. Tidsk., Bd. 14, S. 168–175.

3) Coulter, J. M. 1897. Contribution to the life-history of *Lilium philadelphicum*. Bot. Gaz., Vol. 23, p. p. 412–422.

4) Coulter & Chamberlain. 1903. Morphology of Angiosperms. New York.

5) Dahlgren, K. V. O. 1924. Studien über die Endospermbildung der Kompositen. Sv. Bot. Tidsk., Bd. 18, S. 177–203.

6) Ernst, A. 1901. Beiträge zur Kenntnis der Entwicklung des Embryosackes und des Embryos von *Tulipa Gesneriana*. Flora, Bd. 88, S. 37–77.

7) Gäumann, E. 1919. Studien über die Entwicklungsgeschichte einiger Saxifragales. Rec. d. Trav. bot. Néerlandais, Vol. 16, p. p. 283–322.

8) Ikeda, T. 1902. Studies in the physiological functions of the antipodals and related phenomena of the fertilization in Liliaceae. I. *Tricyrtis hirta*. Bull. Coll. Agr. Univ. Tokyo, Vol. 5, p. p. 41–71.

9) Ishikawa, M. 1916. A list of the number of chromosomes. Bot. Mag. Tokyo, Vol. 30, p. p. 404–448.

10) Palm, B. 1915. Studien über Konstruktionstypen und Entwicklungswege des Embryosackes der Angiospermen. Inaug. Diss. Stockholm.

11) Palm, B. 1922. Das Endosperm von *Hypericum*. Sv. Bot. Tidsk., Bd. 16, S. 60–68.

12) Robbins, W. W. & Borthwick; H. A. 1925. Development of the seed of *Asparagus officinalis*. Bot. Gaz., Vol. 80, p. p. 426–438.

13) Samuelsson, G. 1913. Studien über die Entwicklungsgeschichte der Blüten einiger Bicornes-Typen. Sv. Bot. Tidsk., Bd. 7, S. 97–188.

14) Schaffner, J. H. 1901. A contribution to the life-history and cytology of *Erythronium*. Bot. Gaz., Vol 31, p. p. 369–387.

15) Schnarf, K. 1925. Kleine Beiträge zur Entwicklungsgeschichte der Angiospermen. V. Über zwei kritische Fälle der Endospermentwicklung (*Verbena* und *Triglochin*). Öst. Bot. Zeits., Nr. 1–3, S. 40–50.

16) Schürhoff, P. N. 1924. Die Haploidgeneration der Blütenpflanzen. Bot. Jahrb. (Engler), Bd. 59, S. 198–293.

17) Wettstein, R. 1924. Handbuch der systematischen Botanik. Dritte, Umgearbeitete Auflage. Leipzig und Wien.

Effect of Heat on Phototaxis in *Allolobophora foetida* (SAV.) and its Analysis Based upon the Antagonistic Relation between the Functionings of the Brain and of the Ventral Nerve Cord.

BY

EKITARO NOMURA and SHINRYO OHFUCHI.

"Die Wärme," states STRASBURGER (1878), "übt meist einen Einfluss auf die photometrische Stimmung der Schwärmer. Durch steigende Temperatur werden sie im Allgemeinen lichtholder, durch sinkende lichtscheuer gemacht." Since then the reversibility of the ordinary behavior of organisms by means of heat or temperature has been investigated by a number of students.

1891 MASSART (*fide* MAST, 1907) found that phototaxis in *Chromulina*, a flagellate, was positive at 20°C. and negative at 5°C.

1893 LOEB observed that positive phototaxis in *Polygordius* larvae was changed to negative by a change in temperature from 24° to 29°C., and that negative phototaxis in marine copepods was changed to positive by a decrease, and positive phototaxis to negative by an increase of temperature.

1905 HOLMES observed that raising the temperature tended to accentuate positive phototaxis in *Ranatra fusca* and lowering it tended to produce the negative reaction. "Definite proof," write CROZIER and FEDERIGHI (1924a), however, "is obtained that in the phototropic orientation of *Ranatra* the relative postures of the appendages, resulting in a bilateral difference of effective stroke in swimming, is the mechanism of orientation; the frequency of locomotor movements is the same on the two sides of the body."

1907 MAST found that change in temperature did not induce reversal of phototaxis in *Volvox globator*. "It seems strange," writes MAST, "that organisms so nearly alike as *Chromulina*, *Volvox* and swarm-spores should be affected so differently by change in temperature."

1915 ALLEN found in *Planaria dorotocephala* that the reversal of rheotaxis from positive to negative could be induced by sudden changes in temperature.

1918 KANDA studied geotaxis in *Paramecium caudatum* and found that the so-called reversibility of negative geotaxis by means of changing temperature was extremely doubtful.

1919 KANDA succeeded in producing a reversal of the positive phototaxis in *Arenicola* larvae by means of raising and lowering the temperature of sea-water. According to him, high (31°C.) and low (11°C.) temperatures showed the same reversing effect from positive to negative phototaxis.

1924 CROZIER and FEDERIGHI concluded, in the study of *Limax maximus*, that the expression of the phototropic orienting tendency, with illumination constant, was greatly influenced by the temperature.

1925 HESS studied phototaxis in *Lumbricus terrestris*. "Excessive stimulation, by other means than light," states HESS, "may slightly increase the percentage of positive reactions of earthworms to a given illumination."

The object of the present investigation was to ascertain what kind of phototaxis *Allolobophora foetida* (SASAKI, 1924) would show when it was exposed to comparatively high temperatures, and to note the differential influence of heat on the brain and the ventral nerve cord of this species.

For the purpose of investigation two sets of experiments were carried on, one during the middle part of August and the other during the latter part of December, in a dark room of our laboratory.

In the experiments, many little PETRI dishes, a copper box, an electric lamp of 500 watts, and a bottle containing a 1 per cent. aqueous solution of methylene blue were prepared. The PETRI dishes, as well as the covers, were painted black with enamel paint. The top of the copper box was made horizontal, 40 cm. by 30 cm., having in two of the corners, an opening, one for a thermometer and the other for the escape of vapor. The box was filled with water and heated from below. The electric lamp was used as a light source and was put at a distance of two meters and at an elevation angle of 25° from the middle of the top of the copper box.

On one side of the top of the copper box, near its margin, the PETRI dishes and the bottle of methylene blue were arranged, the other wider side being left for the purpose of spreading a well moistened paper there. The water in the copper box was regulated to keep a constant temperature of 35°C., so that it might be presumed that the temperature of the air in the PETRI dishes, of the solution in the bottle and of the paper containing water was 33°C.

Each worm was washed in drinking water and then was put into a PETRI dish for the purpose of exposing it to the heat. The dish was covered very quickly to prevent the dispersion of the heat. The worm was kept in the PETRI dish for a definite duration and then, after the solution of methylene blue was applied, was placed on the moistened paper for describing its own track, its body being straightened at right angles to the light rays coming from the electric lamp.

The definitions of positive and negative phototaxis, of positive and negative orientation, of the average, positive, and negative occupied angle, of the average, positively, and negatively moved angle, as well as of the given angle, were made according to NOMURA (1926). In the present investigation the magnitude of the given angle was invariably 90°. Let the total number of individuals which were tested be t, the number of individuals which showed positive orientation or occupied angles smaller than 90° be p, and the number of individuals which showed negative orientation or occupied angles larger than 90° be n. Then

$$p+n=t$$

$$\text{Average occupied angle}=\frac{\text{The sum of the angles occupied by } t \text{ individuals}}{t}$$

$$\text{Positive occupied angle}=\frac{\text{The sum of the angles occupied by } p \text{ individuals}+90^\circ n}{t}$$

$$\text{Negative occupied angle}=\frac{\text{The sum of the angles occupied by } n \text{ individuals}+90^\circ p}{t}$$

$$\text{Average occupied angle}-\text{Positive occupied angle}+90^\circ=\text{Negative occupied angle.}$$

The direction or the orientation of the crawl was determined at two points of the track: one at the point of the track intersected by the circumference of a circle of 5 cm. radius (Fig. 1, A) and the other at the point of the track intersected by the circumference of a circle of 10 cm. radius (Fig. 1, B) the common center being located at the point of origin of the crawl (Fig. 1, 0). The former angle is called for convenience the *5 cm. angle* and the latter the *10 cm. angle.* When the worm crawled forward the point of origin of the crawl was located at the anterior end and when it crawled backward at the posterior end of the body.

FIG. 1.

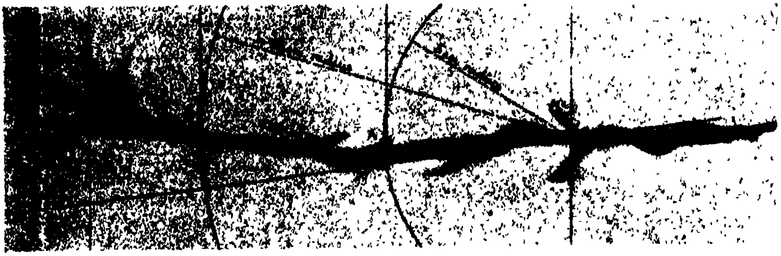


Fig. 1. A track of a forward crawling individual, making the 5 cm. occupied angle 98° and the 10 cm. occupied angle 84°. The direction of light rays is denoted by the vertical lines. ⅓ of natural size.

When the worms were exposed to the heat (? 33°C.) for a longer duration, they showed wriggling or squirming movements on the paper and sometimes orientations by "trial and error" (JENNINGS, 1906), elongating or swinging the anterior or the posterior end of the body (Fig. 1). When the worms were exposed to the heat for a duration longer than 45 minutes, most of them could not crawl for a requisite distance. "A rise in temperature of 10 degrees (i. e., 18°-28°C.) above that in which *Allolobophora foetida* lives," writes Smith (1902), "does not cause the retreat of the worms. A rise from 29° to 35° causes retreat, and violent squirming if the animal is retained in this temperature, while a rise from 35° to 40° is fatal, although such a temperature rarely causes progressive crawling."

The arrangement of subjects is as follows:

Summer Experiments.
  The movements of unoperated worms.
  The movements of operated worms.
  The changes of negativity in the brain.
Winter Experiments.
  The movements of unoperated worms.
  The movements of operated worms.
  The changes of negativity in the brain.
Summary and Conclusions.

Summer Experiments.

The temperature in the dark room was 29.5°C. and the worms were kept in this room for at least one hour previous to trial to become adapted to the temperature.

Two kinds of experiments were carried on, one to test the movements of the worms from which the anterior 4–5 segments were removed, and the other to test the movements of the worms which were not operated upon.

THE MOVEMENTS OF UNOPERATED WORMS.

The tests in which worms were exposed individually to the heat (? 33°C.) for a duration of 0, 1, 5, 10, 15, 20, 25, 30, 35, or 40 minutes were carried on and for each test 50 worms were used.

The orienting movements of the worms (that is directly the phototactic movements in the present case) are shown in Tables 1 and 2.

TABLE 1.

Duration of exposure	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0 min.	3	7	40	2	5	43
1 min.	12	8	30	10	5	35
5 min.	20	8	22	12	10	28
10 min.	20	12	18	16	14	20
15 min.	21	14	15	19	14	17
20 min.	24	15	11	26	10	14
25 min.	20	17	13	19	15	16
30 min.	18	20	12	20	11	19
35 min.	15	14	21	17	9	24
40 min.	16	12	22	12	16	22

In Table 1 the frequency distribution of the worms is given in absolute numbers in the angular sections 0°–80°, 81°–99° and 100°–180°. In this table it may be thought in general that the number of positively orienting individuals is given in the section 0°–80°, the number of negatively orienting individuals in the section 100°–180°, and the number of indistinctly or indifferently orienting individuals in the section 81°–91°. The following facts may be seen in the table:

1. In both the 5 cm. and the 10 cm. angles, the increase of the

duration of exposure from 0 to 20 minutes causes the decrease of the number of negatively orienting and the increase of the number of positively orienting individuals, while the increase of the duration above 20 minutes tends to cause the increase of the number of negatively orienting and the decrease of the number of positively orienting individuals.

2. In both the 5 cm. and the 10 cm. angles, at the durations of exposure of 0, 1, 5, and 10 or 15 minutes the number of negatively orienting individuals is greater than the number of positively orienting individuals, at the durations of exposure of 10 or 15, 20, 25, and 30 minutes it becomes smaller, and at the durations of exposure of 35 and 40 minutes it becomes greater again.

In Table 2 the changes in the magnitude of the occupied angles are shown and from this table Fig. 2 was plotted in order to see easily the state of the changes. The following facts may be seen in Fig. 2:

1. In both the 5 cm. and the 10 cm. angles, at the durations of exposure of 0, 1, 5, and 10 or 15 minutes the magnitude of the negatively moved angle is larger than the magnitude of the positively moved angle, at the durations of 15, 20, 25, and 30 minutes it becomes smaller and at the durations of 35 and 40 minutes it becomes larger again, consequently the average moved angle shows negativity at a duration of exposure from 0 to 10 or 15 minutes, positivity at a duration from 15 to 30 minutes and again negativity at a duration longer than 30 minutes.

2. The magnitude of the negatively moved angle decreases retardatively according to the prolongation of the duration of exposure from 0 to 10 minutes, while it shows no notable alterations at durations from 10 to 30 minutes, though finally it increases for prolongations above 30 minutes.

3. The magnitude of the positively moved angle tends to increase acceleratively according to the prolongation of the duration of exposure from 0 to 20 minutes, while it decreases somewhat retardatively for prolongations above 20 minutes.

4. In consequence, in relation to the prolongation of the duration of exposure to the heat, the average moved angle shows at first a retardative weakening of negativity which alters to a strengthening

TABLE 2.

Duration of exposure	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 min.	88.64	131.54	132.90	88.78	141.00	142.22
1 min.	81.91	109.72	117.81	82.28	115.13	122.85
5 min.	77.76	95.84	108.08	80.40	103.16	112.76
10 min.	82.28	90.72	98.44	79.98	92.76	102.78
15 min.	77.74	86.48	98.74	76.94	90.72	103.78
20 min.	68.52	77.38	98.86	68.94	81.70	102.76
25 min.	74.10	81.26	97.16	69.64	83.52	103.88
30 min.	79.28	85.72	96.44	75.42	86.32	100.90
35 min.	79.24	96.96	107.72	75.60	96.44	109.84
40 min.	80.76	97.66	106.90	82.78	106.00	113.22

FIG. 2.

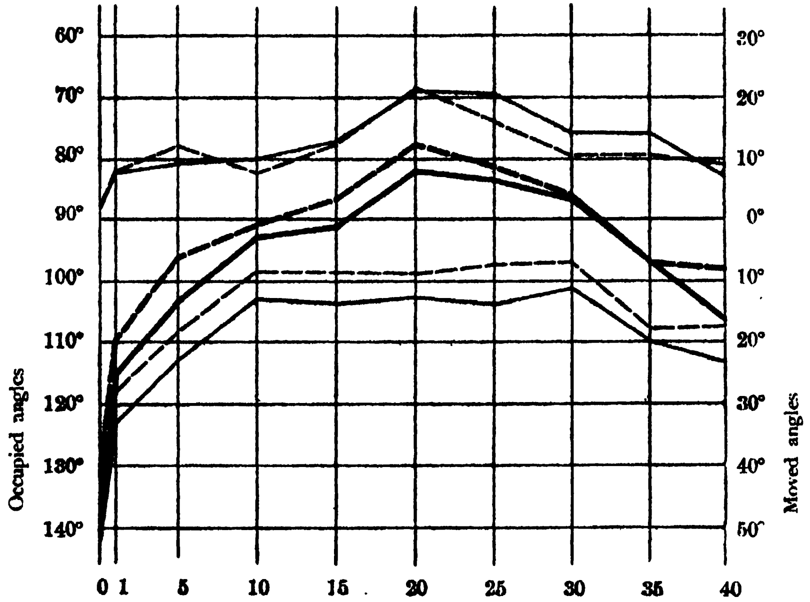


Fig. 2. The given angle 90° or the moved angle 0° is denoted by the horizontal line 90°-0°, and the moved angles upward from this line show the positively and those downward the negatively moved angles. The tracings of the average angles are denoted by the thicker lines, and those of the positive and negative angles by the thinner lines. The tracing of the 5 cm. angles are denoted by the broken lines and those of the 10 cm. angles by the full lines.

positivity, and then an accelerative weakening of the positivity which alters to a strengthening negativity.

From Tables 1 and 2 we may conclude that when the worms are exposed to the heat (? 33°C.) and when they are not operated upon, their orientation alters from negative to positive according to the prolongation of the duration of exposure from 0 to about 20 minutes, but when the duration prolongs beyond this the orientation begins to alter from positive to negative.

THE MOVEMENTS OF OPERATED WORMS.

Five tests were carried on at durations of exposure of 0, 10, 20, 30, and 40 minutes, and in each test 25 worms were used. The removal of the anterior 4-5 segments was made after exposing the worm to the heat (? 33°C.) for a definite duration.

The absolute numbers of forward and backward crawling individuals are given in Table 3. In the table it may be seen that the number of forward crawling individuals tends to decrease with the prolongation of the duration of exposure, while that of backward crawling individuals tends to increase.

TABLE 3.

Duration of exposure	5 cm. angles				10 cm. angles	
	Forward		Backward		Forward	Backward
	Directly	After posterior elongation	Directly	After anterior elongation		
0 min.	11	4	9	1	16	9
10 min.	12	2	11	0	16	9
20 min.	11	3	10	1	15	10
30 min.	11	2	12	0	13	12
40 min.	8	1	15	1	9	16

The absolute numbers of positively, indifferently, and negatively phototactic individuals are given together in Table 4. In this table the forward crawling individuals which made the occupied angles from 0° to 80°, as well as the backward crawling individuals which made the occupied angles from 180° to 100°, are taken as the positively phototactic individuals; the forward crawling individuals which made the occupied angles from 100° to 180°, as well as the backward crawling individuals which made the occupied angles from 80° to 0° as the negatively phototactic individuals; and the remaining, forward or backward crawling individuals as the indifferently phototactic individuals. The following facts may be seen in the table:

TABLE 4.

	Duration of exposure	Positive Phototaxis			Indifferent Phototaxis			Negative Phototaxis		
		Forward crawling 0°-80°	Backward crawling 180°-100°	Sum	Forward crawling 81°-99°	Backward crawling 99°-81°	Sum	Forward crawling 100°-180°	Backward crawling 80°-0°	Sum
5 cm. angles	5 min.	11	2	13	2	4	6	2	4	6
	10 min.	10	5	15	1	3	4	3	3	6
	20 min.	10	5	15	1	2	3	3	4	7
	30 min.	10	6	16	0	3	3	3	3	6
	40 min.	4	11	15	1	3	4	4	2	6
	Average			14.8			4.0			6.2
10 cm. angles	0 min.	12	2	14	0	2	2	4	5	9
	10 min.	10	3	13	2	0	2	4	6	10
	20 min.	10	3	13	2	1	3	3	6	9
	30 min.	10	6	16	0	1	1	3	5	8
	40 min.	5	11	16	0	3	3	4	2	6
	Average			14.4			2.2			8.4

1. The number of positively, as well as of negatively, phototactic individuals shows no notable alterations at any duration of exposure, and the number of positively phototactic individuals is invariably greater than that of negatively phototactic individuals.

2. The number of individuals which crawled forward toward the light (positively phototactic and positively orienting) tends to decrease with the prolongation of the duration of exposure, while the number of individuals which crawled backward toward the light (positively phototactic, but negatively orienting) tends to increase.

From Tables 3 and 4 we may conclude that when the worms are exposed to the heat and when they are operated upon, the number of forward crawling individuals, as well as of positively phototactic individuals which crawled forward, tends to decrease with the prolongation of the duration of exposure, while the number of backward crawling individuals, as well as of positively phototactic individuals which crawled backward, tends to increase; consequently, the number of positively phototactic individuals invariably surpasses the number of negatively phototactic individuals at any duration of exposure.

The orienting movements of the worms which were operated upon are shown in Tables 5 and 6.

TABLE 5.

Duration of exposure	5 cm. angles			10 cm. angles		
	0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
0 min.	15	6	4	17	2	6
10 min.	15	4	6	16	2	7
20 min.	15	3	7	14	2	9
30 min.	13	3	9	15	1	9
40 min.	6	5	14	8	3	14

In Table 5 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

1. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals (section 0°-80°) tends to decrease with the prolongation of the duration of exposure, while the number of negatively orienting individuals (section 100°-180°) tends to increase.

2. In both the 5 cm. and the 10 cm. angles, at durations of exposure from 0 to 30 minutes, the number of positively orienting individuals is greater than the number of negatively orienting individuals, but at an exposure of 40 minutes the reverse is the case.

In Table 6 the changes in the magnitude of the occupied angles are shown and from this table Fig. 3 was plotted in order to see easily the state of the changes. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, at the durations of exposure of 0, 10, 20, and 30 minutes the magnitude of the positively moved angle is larger than that of the corresponding negatively moved angle, consequently the average moved angle shows the positivity of the worms which were operated upon; but at the exposure of 40 minutes the magnitude of the positively moved angle becomes smaller than that of the corresponding negatively moved angle, consequently

the average moved angle shows the negativity of even the worms which were operated upon.

TABLE 6.

Duration of exposure	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 min.	57.84	64.40	95.56	47.84	60.40	102.56
10 min.	59.22	67.30	99.08	52.04	64.84	102.80
20 min.	62.84	73.20	100.36	51.44	69.76	108.32
30 min.	64.68	80.80	106.12	55.32	76.52	111.20
40 min.	81.02	102.16	111.14	75.66	102.36	116.70

FIG. 3.

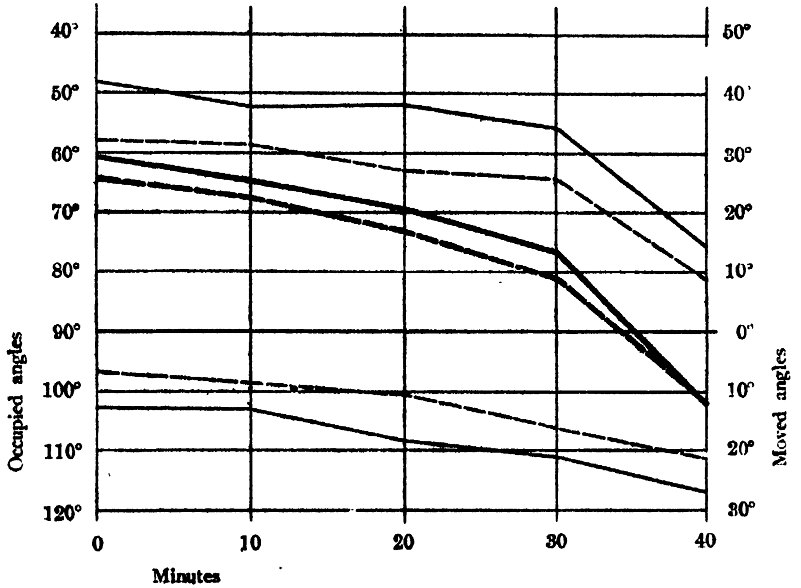


Fig. 3. The plot is made in the same way as in Fig. 2.

2. In both the 5 cm. and the 10 cm. angles, the magnitude of the negatively moved angle or the negativity of the worms increases acceleratively with the prolongation of the duration of exposure, while the magnitude of the positively moved angle or the positivity of the

worms decreases acceleratively. In consequence, the average moved angle shows that positive orientation in the operated worms becomes weakened in relation to the prolongation of the duration of exposure, till finally it changes to negative orientation at durations of exposure longer than about 35 minutes.

From Tables 5 and 6 we may conclude that when the worms are exposed to the heat (? 33°C.) and when they are operated upon, their degree of positive orientation becomes weaker according to the prolongation of the duration of exposure, and, in turn, the degree of negative orientation becomes stronger. Then it follows that the higher temperature causes an inhibition in the ventral nerve cord of the positively orienting functioning which is stronger than the inhibition of the negatively orienting functioning, consequently the degree of negative orientation in the ventral nerve cord becomes strengthened in relation to the prolongation of the duration of exposure, while the degree of positive orientation becomes weakened.

THE CHANGES OF NEGATIVITY IN THE BRAIN.

According to NOMURA (1926) the photic orientation of *Allolobophora foetida* is determined by the antagonism between the functionings of the brain and of the ventral nerve cord.

Now let the degree of photic orientation of the worms be A, the degree of negative orientation which is exhibited by the functioning of the brain be N, and the degree of positive orientation which is exhibited by the functioning of the ventral nerve cord be P. Then from the antagonistic relation we find the equation

$$A - P + 90^\circ = N,$$

in which 90° is the magnitude of the given angle in the present experiments.

The values of A are directly the magnitudes of the average occupied angles made by the worms which were not operated upon, and the values of P may be assumed in probable approximation to be the magnitudes of the average occupied angles made by the worms which were operated upon. Then from the data given for the operated and the unoperated worms, we may calculate the probable values of N.

In Table 7 the values of P, A and N are given together, and from

TABLE 7.

Duration of exposure	5 cm. angles in degrees			10 cm. angles in degrees		
	P	*A*	*N*	*P*	*A*	*N*
0 min.	64.40	131.54	157.14	60.40	141.00	170.60
10 min.	67.80	90.72	118.42	64.84	92.76	117.92
20 min.	73.20	77.38	94.18	69.76	81.70	101.94
30 min.	80.80	85.72	94.92	76.52	86.32	99.80
40 min.	102.16	97.66	85.50	102.36	106.00	93.64

FIG. 4.

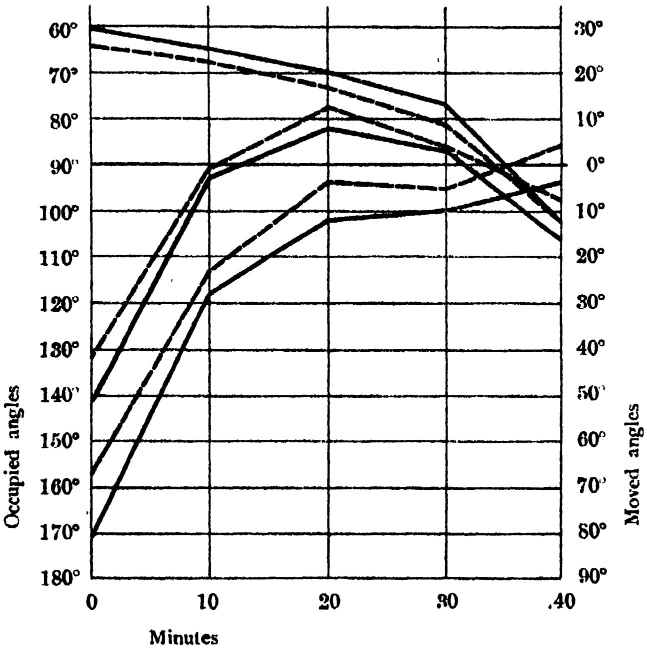


Fig. 4. The tracings of the 5 cm. angles are denoted by the broken lines and those of the 10 cm. angles by the full lines.

this table Fig. 4 was plotted in order to see easily the state of the changes. It may be seen in the figure that when the worms are exposed to the heat (? 23°C.) the degree of negative orientation which is exhibited by the brain becomes weaker retardatively with the prolongation of the duration of exposure, till finally its degree is 0 at the exposure of about 40 minutes.

It is notable that, following the prolongation of the duration of exposure from about 20 to 40 or more minutes, the degree of negative orientation in the unoperated worms becomes stronger, because the degree of negative orientation becomes stronger in the ventral nerve cord, notwithstanding the degree of negative orientation becoming weaker in the brain.

Winter Experiments.

The temperature in the dark room was 7.8°–9.4°C. and the worms were kept in this room for at least one hour previous to trial to become adapted to the temperature.

THE MOVEMENTS OF UNOPERATED WORMS.

Five tests were carried on and at each test 50 worms were used after being exposed individually to the heat (? 33°C.) for a duration of 0, 10, 20, 30, or 40 minutes.

The orienting movements of the worms are shown in Tables 8 and 9.

In Table 8 the frequency distribution of the worms is shown in absolute numbers in the angular sections 0°–80°, 81°–99°, and 100°–180°. The following facts may be seen in the table:

TABLE 8.

Duration of exposure	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0 min.	12	8	30	12	7	31
10 min.	13	9	28	13	8	29
20 min.	13	12	25	14	8	28
30 min.	14	14	22	22	5	23
40 min.	24	6	20	25	5	20

1. In both the 5 cm. and the 10 cm. angles, the increase of the number of minutes causes the increase of the number of positively orienting individuals and the decrease of the number of negatively orienting individuals.

2. In both the 5 cm. and the 10 cm. angles, at a shorter duration of exposure the number of negatively orienting individuals is greater

than that of positively orienting ones, but at an exposure of 40 minutes the reverse is the case.

TABLE 9.

Duration of exposure	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0 min.	83.58	97.66	104.08	80.84	108.82	117.98
10 min.	80.52	98.28	107.76	80.76	107.68	116.92
20 min.	81.32	100.92	109.60	80.40	106.96	116.56
30 min.	77.58	97.96	110.38	68.60	90.92	112.42
40 min.	72.98	89.14	106.16	66.16	86.80	110.64

FIG. 5.

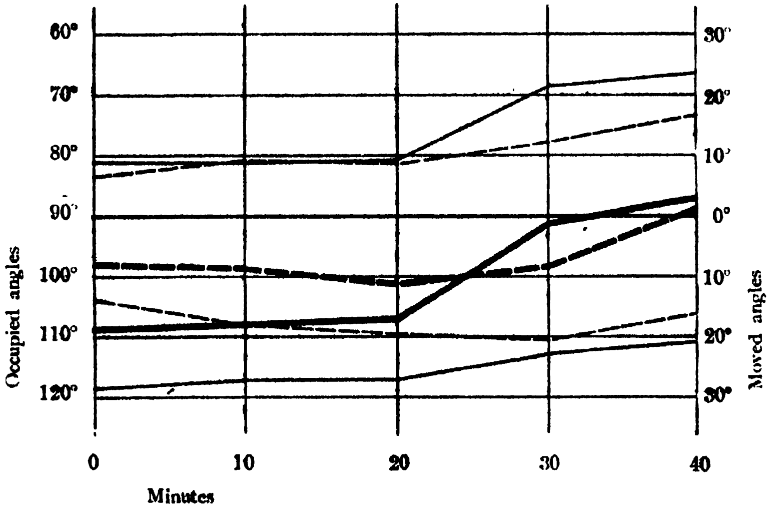


Fig. 5. The plot is made in the same way as in Fig. 2.

In Table 9 the changes in the magnitude of the occupied angles are shown and from this table Fig. 5 was plotted. The following facts may be seen in the figure:

1. Save the negative 5 cm. angle, all the other occupied angles tend to decrease the magnitude with the increase of the number of minutes.

2. In both the 5 cm. and the 10 cm. angles, at a duration of exposure shorter than 20 minutes the negatively moved angle is larger than the positively moved angle, but at a duration longer than 20 minutes the nagetively moved angle tends to become equal to the positively moved angle. In consequence, the average moved angle shows an accelerative weakening of negativity with the prolongation of the duration of exposure.

From Tables 8 and 9 we may conclude that when the worms are exposed to the heat (? 33°C.) and when they are not operated upon, their degree of negative orientation becomes weakened acceleratively according to the prolongation of the duration of exposure, while the degree of positive orientation becomes strengthened acceleratively.

THE MOVEMENTS OF OPERATED WORMS.

Five tests were carried on at durations of exposure of 0, 10, 20, 30, and 40 minutes, and in each test 25 worms were used. The removal of the anterior 4–5 segments was made after exposing them individually to the heat (? 33°C.) for a definite duration.

TABLE 10.

Duration of exposure	5 cm. angles				10 cm. angles	
	Forward		Backward		Forward	Backward
	Directly	After posterior elongation	Directly	After anterior elongation		
0 min.	24	1	0	0	25	0
10 min.	16	4	4	1	22	3
20 min	18	2	5	0	22	3
30 min.	20	1	4	0	22	3
40 min.	20	1	2	2	22	3

The absolute numbers of forward and backward crawling individuals are given in Table 10. In the table it may be seen that when the worms are exposed to the heat the number of backward crawling individuals increases; that the number of forward crawling individuals is greater than the number of backward crawling individuals; and that the ratio between the forward and the backward crawling individuals is nearly constant at any duration of exposure.

TABLE 11.

	Duration of exposure	Positive Phototaxis			Indifferent Phototaxis			Negative Phototaxis		
		Forward crawling 0°–80°	Backward crawling 180°–100°	Sum	Forward crawling 81°–99°	Backward crawling 99°–81°	Sum	Forward crawling 100°–180°	Backward crawling 80°–0°	Sum
5 cm. angles	0 min.	24	0	24	1	0	1	0	0	0
	10 min.	19	1	20	1	0	1	0	4	4
	20 min.	18	2	20	1	0	1	1	3	4
	30 min.	16	0	16	4	2	6	1	2	3
	40 min.	18	3	21	1	0	1	2	1	3
10 cm. angles	0 min.	24	0	24	0	0	0	1	0	1
	10 min.	21	0	21	0	0	0	1	3	4
	20 min.	19	0	19	1	1	2	2	2	4
	30 min.	20	1	21	1	0	1	1	2	3
	40 min.	18	2	20	2	0	2	2	1	3

The absolute numbers of positively, indifferently, and negatively phototactic individuals are given together in Table 11 in the same way as Table 4. I am not able to find any regular changes in this table.

The orienting movements of the worms which were operated upon are shown in Tables 12 and 13.

TABLE 12.

Duration of exposure	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0 min.	24	1	0	24	0	1
10 min.	23	1	1	24	0	1
20 min.	21	1	3	21	2	2
30 min.	18	6	1	22	1	2
40 min.	19	1	5	19	2	4

In Table 12 the frequency distribution of the worms is shown in absolute numbers. The following facts may be seen in the table:

1. In both the 5 cm. and the 10 cm. angles, the number of positively orienting individuals (section 0°–80°) tends to decrease with the prolongation of the duration of exposure, while that of negatively orienting individuals (section 100°–180°) tends to increase.
2. In both the 5 cm. and the 10 cm. angles, the number of

positively orienting individuals is greater than that of negatively orienting individuals.

TABLE 13.

Duration of exposure	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	·Average	Negative
0 min.	26.96	26.96	90.00	21.92	24.36	92.44
10 min.	33.60	35.36	91.76	24.20	25.20	91.00
20 min.	43.12	46.12	93.00	33.48	37.40	93.92
30 min.	48.20	49.48	91.28	29.82	31.96	92.64
40 min.	47.52	54.84	97.32	32.40	42.60	100.20

FIG. 6.

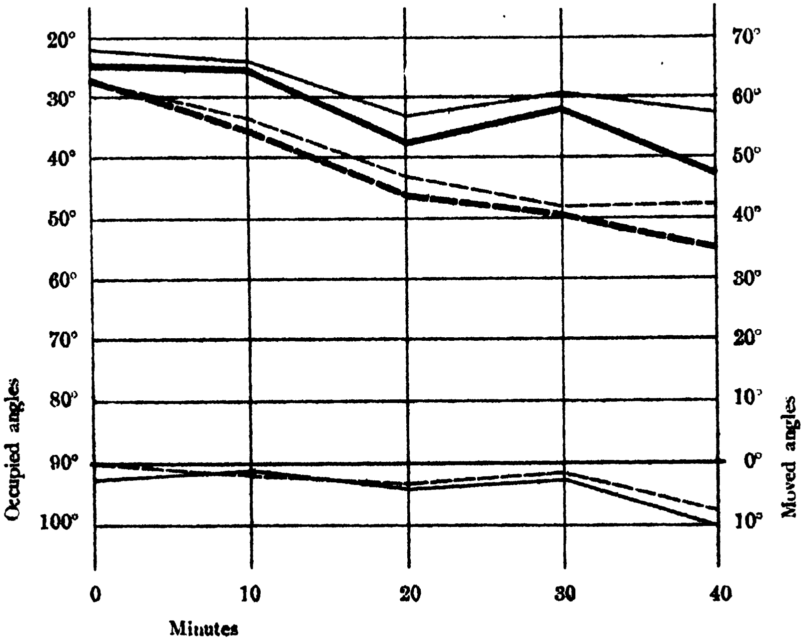


Fig. 6. The plot is made in the same way as in Fig. 2.

In Table 13 the changes in the magnitude of the occupied angles are shown and from this table Fig. 6 was plotted in order to see easily the state of the changes. The following facts may be seen in the figure:

1. In both the 5 cm. and the 10 cm. angles, all the occupied angles tend to increase in magnitude with the increase of the number of minutes.

2. In both the 5 cm. and the 10 cm. angles, the positively moved angle is larger than the negatively moved angle at any duration of exposure.

From Tables 12 and 13 we may conclude that when the worms are exposed to the heat (? 33°C.) and when they are operated upon, their degree of positive orientation becomes weaker according to the prolongation of the duration of exposure, and in turn the degree of negative orientation becomes stronger.

THE CHANGES OF NEGATIVITY IN THE BRAIN.

The magnitudes of the average occupied angle *A* which were made by the unoperated worms, the magnitudes of the average occupied angle *P* which were made by the operated worms, and the calculated magnitudes of *N* which show the degree of negative orientation in the brain, are given together in Table 14, and from this table Fig. 7 was plotted in order to see easily the state of the changes.

In Fig. 14 it may be seen that when the worms are exposed to the heat (? 33°C.) the degree of negative orientation which is exhibited by the brain becomes weaker according to the prolongation of the duration of exposure.

TABLE 14.

Duration of exposure	5 cm. angles in degrees			10 cm. angles in degrees		
	P	*A*	*N*	*P*	*A*	*N*
0 min.	26.96	97.66	160.70	24.36	108.82	174.46
10 min.	35.36	98.28	152.92	25.20	107.68	172.48
20 min.	46.12	100.92	144.80	37.40	106.96	159.56
30 min.	49.48	97.96	138.48	31.96	90.92	148.96
40 min.	54.84	89.14	124.30	42.60	86.80	134.20

FIG. 7.

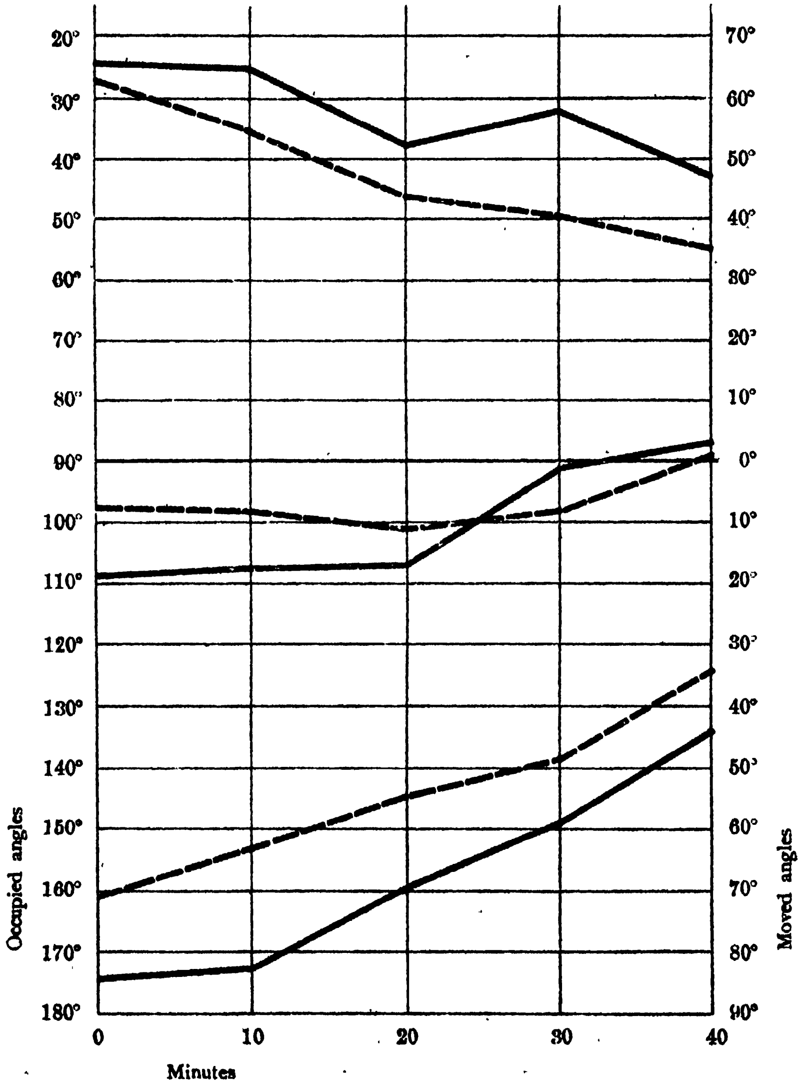


Fig. 7. The plot is made in the same way as in Fig. 4.

Summary and Conclusions.

In comparing Figs. 4 and 7 we may infer that positive orientation is more pronounced in winter than in summer, and that heat (? 33°C. influences phototaxis more strongly in worms from a higher temperature (that is 29.5°C. in August) than in worms from a lower temperature (that is 7.8°-9.4°C. in December).

In combining the results from the experiments in August and in December we may conclude in general as follows:

1. In the ventral nerve cord, heat causes a weakening of the positively orienting functioning and, in turn, a strengthening of the negatively orienting functioning, and an inhibition of forward crawling.

2. In the brain, heat causes a weakening of the negatively orienting functioning.

3. In consequence, in worms heat causes several alterations of phototaxis as well as " trial and error " orientations, crawling backward or forward, by the antagonistic relations between the functionings of the brain and of the ventral nerve cord.

January 20, 1926.

LITERATURE CITED.

1) Allen, G. D. 1915. Reversibility of the reactions of *Planaria dorotocephala* to a current of water. Biol. Bull., Vol. 29, pp. 111-128.

2) Crozier, W. J. and Federighi, H. 1924. Phototropic circus movements of *Limax* as affected by temperature. Jour. Gen. Physiol., Vol. 7, pp. 151-169.

3) Crozier, W. J. and Federighi, H. 1924a. The phototropic mechanism in *Ranatra*. Ibid., Vol. 7, pp. 215-220.

4) Hess, W. H. 1924. Reactions to light in the earthworm, *Lumbricus terrestris* L. Jour. Morph. Physiol., Vol. 39, pp. 515-542.

5) Holmes, S. J. 1905. The reaction of *Ranatra* to light. Jour. Comp. Neurol., Vol. 15, pp. 303-349.

6) Jennings, H. S. 1906. Modifiability in behavior. II Factors determining direction and character of movement in the earthworm. Jour. Exp. Zool., Vol. 3, pp. 435-455.

7) Kanda, S. 1918. Further studies on the geotropism of *Paramecium caudatum*. Biol. Bull, Vol. 35, pp. 108-119.

8) Kanda, S. 1919. On the reversibility of the heliotropism of *Arenicola* larvae by chemicals. Ibid., Vol. 36, pp. 149-166.

9) Loeb, J. 1893. Über künstliche Umwandlung positiv heliotropischer Thiere in negativ heliotropische und umgekehrt. Pflüger's Archiv, Bd. 54, S. 81-107.

10) Mast, S. O. 1907. Light reactions in lower organisms. II *Volvox globator*. Jour. Comp. Neurol., Vol. 17, pp. 99-180.

1) NOMURA, E. 1926. Effect of light on the movements of the earthworm, *Allolobophora foetida* (SAV.). Science Reports Tohoku Imp. Univ., Biology, Vol. 1, pp. 293-409.

12) SASAKI, K. 1924. *Allolobophora foetida* (SAV.) in North Japan. Ibid., Vol. 1, pp. 89-90.

13) SMITH, A. C. 1902. The influence of temperature, odors, light and contact on the movements of the earthworm, *Allolobophora foetida*. Amer. Jour. Physiol., Vol. 3, pp. 435–455.

14) STRASBURGER, E. 1878. Wirkung des Lichtes und der Wärme auf Schwärmsporen. Jena. Zeit., N. F., Bd. 12, S. 551-625.

On the Fertilizin of the Oyster, *Ostrea circumpicta*.*

BY

ARATA TERAO.

(Imperial Fisheries Institute, Fukagawa, Tokyo.)

Now that the fact of the specificity of egg secretion of several marine animals in agglutinating spermatozoa of the same and other species is fairly well established, the question naturally suggests itself whether or not this specificity can be expressed, at least in some forms, in terms of degree of concentration of egg secretion employed. In the following paragraphs is given a preliminary account of the influence of successively diluted solutions of egg secretion of the oyster, *Ostrea circumpicta* PILSBRY, upon sperm suspensions of the same, another mollusc, *Arca ocellata* REEVE, and two echinoderms, *Luidia quinaria* v. MARTENS and *Toxocidaris tuberculatus* (LAMK).

It is my pleasant duty to return herewith my hearty thanks to Professor HATAI, Director of the Asamushi Marine Biological Station, for the priviledges kindly extended to me there. I also fully appreciate the benefit of discussion of the whole problem with Assistant Professor SHICHIROKU NOMURA, who has already made a valuable contribution in this field (1924).

MATERIAL AND METHOD.

A mixture of 0.55 cc. of eggs of the oyster and 9 cc. of sea-water was allowed to stand for 20 minutes at room temperature, centrifuged for 5 minutes, and filtered. This filtrate was used as standard egg-water. Dilution was made successively as low as 1/10000000 of the standard. About 0.05 cc. of 1% sperm suspension of each species was placed on slides and to each was added by a capillary a small drop of egg-water of desired concentration. It is clearly confessed that a source of error exists in such a crude metod, if the degree of concentration be of much concern in the experimentation, but I think it is admissible that the error is not so great as to make the successive dilutions quite meaningless. Thus I put on record the following results as a first approximation to a quantitative interrelation.

---

* A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

ISO-AGGLUTINATION.

An immediate activation and aggregation are followed by remarkable agglutination even with such a low concentration as 1/500000 of the standard, as shown in Table 1. The agglutination did not take place so promptly with 1/1000000 of the original. It took much more time for the occurrence of the phenomenon with 1/10000000 of the same. Although the effect of more diluted egg-water was not tested, the last mentioned probably lies near the limit of effectiveness, as may be judged by other experiments. The agglutinated mass of spermatozoa, whose heads became a little swollen, formed a three-dimensioned pinwheel, without, however, reversely set spermatozoa in the marginal zone. The reversal of the phenomenon took place after a while.

TABLE 1.

Iso-agglutination of spermatozoa of the oyster.

Concentration of egg-water	Activation	Aggregation	Agglutination
1	Immediate and intense	Immediate and conspicuous	Conspicuous and prompt
1/100	"	"	"
1/1000	"	"	"
1/10000	"	"	"
1/100000	"	"	"
1/500000	"	"	"
1/1000000	"	"	Conspicuous but not prompt
1/10000000	Immediate but not intense	Immediate but not conspicuous	Inconspicuous and a little delayed

HETERO-AGGLUTINATION.

Hetero-agglutination of spermatozoa of the bivalve mollusc, *Arca ocellata*, by egg-water of the oyster was quite the same as iso-agglutination of spermatozoa of the oyster with a concentration as low as 1/10000 of the original. The spermatozoa of *Arca*, however, did not give a typical agglutination reaction but was found "clumping" with 1/100000 concentration of the standard egg-water, and such a low concentration as 1/500000 of the standard could not produce any reaction of the spermatozoa in question except activation. The results of the observations are summarized in Table 2.

TABLE 2.

Hetero-agglutination of spermatozoa of *Arca* by egg-water of the oyster.

Concentration of egg-water	Activation	Aggregation	Agglutination
1	Immediate and intense	Immediate and conspicuous	Conspicuous and prompt
1/100	"	"	"
1/1000	"	"	"
1/10000	"	"	"
1/100000	"	Immediate but not conspicuous	Clumping
1/500000	Immediate but not intense	No	No

From Tables 3 and 4 it will be seen that spermatozoa of the starfish, *Luidia quinaria*, and of the sea-urchin, *Toxocidaris tubeculatus*, behaved similarly in response to the egg-water of the oyster. However, the minimum concentration of the fluid necessary for production of

TABLE 3.

Hetero-agglutination of spermatozoa of *Luidia* by egg-water of the oyster.

Concentration of egg-water	Activation	Aggregation	Agglutination
1	Immediate but not intense	Inconspicuous, not immediate	Inconspicuous, a little delayed
1/100	"	"	"
1/1000	"	"	"
1/10000	"	"	"
1/100000	"	"	"
1/500000	"	"	"
1/1000000	"	"	"
1/10000000	"	"	Slight and delayed

TABLE 4.

Hetero-agglutination of spermatozoa of *Toxocidaris* by egg-water of the oyster.

Concentration of egg-water	Activation	Aggregation	Agglutination
1	Immediate but not intense	Inconspicuous, not immediate	Inconspicuous, a little delayed
1/100	"	"	"
1/1000	"	"	"
1/10000	"	"	"
1/100000	"	"	"
1/500000	"	"	"
1/1000000	"	"	"
1/10000000	"	"	No agglutination

agglutination was lower in the starfish than in the sea-urchin. The reactions were not so prompt. The heads of spermatozoa were greatly swollen and agglutinated, resulting in mass coagulation which never reversed. It may be mentioned that the spermatozoa of the two echinoderms were often found, when put under the influence of the egg-water of the oyster, rotating in pairs round each other. Such pairs were, so to speak, like binary stars going round a common centre of gravity.

DISCUSSION.

From the fact that the egg-water of the oyster agglutinated spermatozoa of the same species, as well as of *Arca*, in the same manner except that a difference in the limit of its effectiveness exists, the specificity of the egg-water can be expressed in terms of degree of concentration. Thus the standard egg-water I employed may be said, according to LILLIE's standardization, to contain more than 10000000 units for iso-agglutination and about 100000 units for hetero-agglutination of spermatozoa of *Arca*. But since hetero-agglutination of spermatozoa of the two echinoderms by the egg-water was of a quite different character, the specificity of the water can no longer be given quantitatively.

As far as we are aware, identity of hetero-agglutination and iso-agglutination in quality was reported once by Sampson (1922) in spermatozoa of *Katherina*. The other cases of hetero-agglutination put on record are utterly different from iso-agglutination, being toxic in nature even with the sexual elements of closely associated species. Two explanations for such a difference may be given. First, we can assume two types of hetero-agglutination to account for it, one identical with iso-agglutination in essentials and exhibiting such specificity as is comparable to that of immune blood-serum, and the other qualitatively different from iso-agglutination. Second, we can argue that even such an instance of hetero-agglutination as is apparently identical with iso-agglutination is really almost inconceivably different from the latter in essential characters and thus the specificity of the egg-water is somewhat comparable to that of luciferin-luciferase reaction of bioluminescence. Although we are not yet in a position to decide between the two alternatives, the predominence of evident cases of toxic reaction in hetero-agglutination makes the second much more probable.

Aggregation of spermatozoa as induced by egg-water has been said to occur where a grade of concentration of the latter exists. Truly it does. But I would like to point out another factor for the production of the phenomenon in question. The heads of spermatozoa when changed to be agglutinizable in response to egg-water cannot be without effect upon each other even before the final agglutination. I should think the viscosity of the surrounding medium is augmented at that time, with result that the spiral motion of the spermatozoa is slackened, and contributes something to aggregation. The binaries-like motion already referred to may be looked upon as a result of such mutual influence. The appearance of reversely set spermatozoa in the marginal zone in pinwheel agglutination of *Katherina*, as reported by Sampson, seems to me also due to such disturbance in the fluid. Viewed from this point, the agglutination may be said to exist at increasing degrees in the series: aggregation, binary rotation, clumping, and pinwheel agglutination.

LITERATURE.

Goto, S. 1914. A descriptive monograph of Japanese Asteroidea. Jour. Coll. Sci. Imp. Univ. Tokyo, Vol. 29, art. 1, pp. 293–300, pl. VII, figs. 104–112.

Glaser, O. C. 1914. A qualitative analysis of the egg secretions and extracts of *Arbacia* and *Asterias*. Biol. Bull., Vol. 26, pp. 367–386. 1921. Fertilization and egg-secretions. Ibid., Vol. 41, pp. 63–72.

IWAKAWA, T. 1915. A hand-list of Japanese bivalves. Dobuts. Zass. (Zool. Mag.), Vol. 27, no. 385, appendix p. 13. 1919. Catalogue of Japanese mollusca in the Natural History Department, Tokyo Imp. Museum, p. 256.

JUST, E. E. 1915. An experimental analysis of fertilization in *Platynereis megalops*. Biol. Bull., Vol. 28, pp. 93–114. 1919. The fertilization reaction in *Echinarachnius parma*. II. The rôle of fertilizin in straight and cross-fertilization. Ibid., Vol. 36, pp. 11–38.

LILLIE, F. R. 1912. The production of sperm iso-agglutinin by ova. Science, N. S., Vol. 36, pp. 527–530. 1913. Studies of fertilization. V. The behavior of the spermatozoa of *Nereis* and *Arbacia* with special reference to egg extractives. Jour. Exp. Zool., Vol. 14, pp. 413–476. 1914. Studies of fertilization. VI. The mechanism of fertilization in *Arbacia*. Ibid., Vol. 16, pp. 523–590. 1915. Sperm agglutination and fertilization. Biol. Bull., Vol. 28, pp. 18–33. 1919. Problems of fertilization. Univ. of Chicago Press. Chaps IV., V., VI. 1921. Studies of fertilization. VIII. On the measure of specificity in fertilization between two associated species of the sea-urchin genus *Strongylocentrotus*. Biol. Bull., Vol. 40, pp. 1–23.

LOEB, J. 1914. Cluster formation of spermatozoa caused by specific substances from eggs. Jour. Exp. Zool., Vol. 17, pp. 123–140.

MORTENSEN, Th. 1914. On the development of some Japanese echinoderms. Preliminary notice. Annot. Zool. Japan., Vol. 8, pp. 543–552

NOMURA, S. 1924 On the agglutinating substance of the egg of the starfish, *Asterina pectinifera*. Sci. Rep. Tohoku Imp. Univ., 4th ser., Biol., Vol. 1, pp. 83–87.

RICHARDS, A. and WOODWARD, A. E. 1915. Note on the effect of X-radiation on fertilizin. Biol. Bull., Vol. 28, pp. 140–148.

SAMPSON, M. M. 1922. Iso-agglutination and hetero-agglutination of spermatozoa. Ibid., Vol. 43, pp. 267–284, pl. 1.

WOODWARD, A. E. 1918. Studies on the physiological significance of certain precipitate from the egg-secretions of *Arbacia* and *Asterias*. Jour. Exp. Zool, Vol. 26, pp. 459–500, pl. 1.

The Influence of Oxygen Tension on the Rate of Oxygen Consumption in *Caudina*.*

By

SHICHIROKU NOMURA.

INTRODUCTORY.

Diverse results have been published as to the relation between the respiratory rate in different forms of animals and the oxygen tension of the surrounding medium. In vertebrates in general and in some invertebrates the respiratory rate is almost independent of the oxygen tension of the medium within a wide range of tension (DAKIN 1925, HENZE 1910, WINTERSTEIN 1908, etc.). In some invertebrates, however, it is much affected by the variation of the oxygen tension (THUNBERG, KROGH), and it has been shown that the rate is proportional to the oxygen tension in some forms (AMBERSON and others). KONOPACKI, on the other hand, reports that the rate of oxygen consumption in the earthworm is proportional to the square root of the oxygen tension of the medium (DAKIN).

KROGH and HENZE maintain the view that the respiratory rate of cold-blooded animals (*e.g.*, *Carcinus*, *Scyllarus*, *Aplysia*, *Eledone*) is independent of the oxygen tension of the surrounding medium except in some of the simpler forms (*e.g.*, *Actinia*, *Anemonia*, *Sipunculus*), where, because of inadequate respiratory or circulatory mechanisms, the rate of oxygen consumption is much affected by the oxygen tension of the surroundings.

The recent work of AMBERSON, MAYERSON, and SCOTT drew our attention again to this problem. They claim to have secured evidence which indicates clearly that HENZE's distinction between the simpler and the more complex invertebrates, with respect to the respiratory function, is by no means absolute. Therefore it is desirable that much be done in this line of investigation.

---

* A contribution from the Marine Biological Laboratory, Asamushi, Aomori Ken. The abstract of this paper was read before the second annual meeting of the Zoological Society of Japan, held in Kyoto, April 5, 1926.

The present paper comprises the result of my experiment on *Caudina chilensis* (J. MÜLLER), a peculiar form of Holothuroidea. This animal has rarely been used for physiological purposes, as far as I am aware, owing to its limited distribution (WINTERSTEIN, 1909, 1912). It is, however, pretty abundant in the inlet of Moura, near the Marine Biological Laboratory at Asamushi. The animal lives in the sandy bottom and can be collected easily. I was fortunate in being able to utilize quite fresh specimens.

It may be of interest to know how the problem above mentioned is to be settled in this peculiar form in connection with the result of AMBERSON and his co-workers and also with the work on this animal which is being carried on by my colleagues and will be published in the near future in this journal.

METHOD.

Ten fresh specimens were allowed to respire in a glass vessel with a narrow mouth, containing 1150 cc. of sea water of a known concentration of oxygen. The surface of the water was covered with a thick layer of liquid paraffine to prevent the diffusion of oxygen from the air, as is usually done in such experiments. The water was continually stirred up with a small glass stirrer, immersed into the water through the paraffine layer and driven by an electric motor, to fascilitate a uniform distribution of oxygen in the medium. The temperature was kept constant, approximately at 10.5°C.

The decrease in the concentration of oxygen in the medium, caused by the respiratory function of the animals, was followed by determining the oxygen content of the sample of water withdrawn from the medium at fixed intervals, by the Winkler's method with a slight modification. For this purpose 15 cc. of water were taken out of the respiratory vessel with a pipette at each interval and 10 cc. thereof were used for analysis. A few cubic centimeters of liquid paraffine were admitted into the pipette before the water was withdrawn, so that paraffine covered the upper surface of water in the pipette and prevented diffusion of oxygen from the air. 0.01 N solution of sodium thiosulphate from a micro-burette was used for the titration of iodine. Such a procedure as described above, using only 10 cc. of water sample, was adopted after it was ascertained that the error of estimation of oxygen lay within a limit easily admissible for the present purpose. The volume of the medium gradually diminished, owing to sampling of water for analysis. To obliterate the difference of result issuing from this change of experimental condition, the time of sampling was appropriately corrected as if the volume had been maintained unchanged.

RESULT OF THE EXPERIMENT.

The numerical data of one case, where the experiment proceeded regularly and satisfactorily, are given in Table I. When the decreasing concentration of oxygen in the medium is plotted against the time, an exponential curve is obtained (Fig. 1), and when the logarithm of oxygen concentration is plotted against time, a straight line is obtained (Fig. 2). This relation indicates that the rate of oxygen consumption at every instant is directly proportional to the oxygen tension in the medium at that instant.

The mathematical expression of this relation is given as follows:

$$-\frac{dC}{dt} = kC \qquad \text{or}$$

$$C_t = C_0 e^{-kt}; \qquad k = \frac{1}{t} \log_e \frac{C_0}{C_t}$$

where C_0 and C_t indicate the concentration of oxygen at time O and t respectively, and k is a constant. e is the base of Napierian logarithms.

TABLE 1.

Decrease in Oxygen Tension in the Medium.

Time in Min. (empirical)	0	120	240	360	480	600	720	1320	1440	1560
Time in Min. (corrected)	0	120	242	365	490	617	745	1398	1530	1664
Thiosulphate,* cc. per 10 cc.	1.11	0.97	0.81	0.69	0.57	0.47	0.43	0.20	0.18	0.14
Oxygen, cc. per liter.	5.74	5.02	4.20	3.56	2.94	2.43	2.22	1.03	0.93	0.72
log O_2	0.758	0.700	0.623	0.551	0.468	0.385	0.346	0.012	$\bar{1}.968$	$\bar{1}.847$
$k \times 10^3$		1.10	1.29	1.31	1.36	1.38	1.26	1.24	1.20	1.24

* 1 cc. of the sodium thiosulphate solution corresponded to 0.0517 cc. of oxygen (0°C. 760 mm. Hg.).

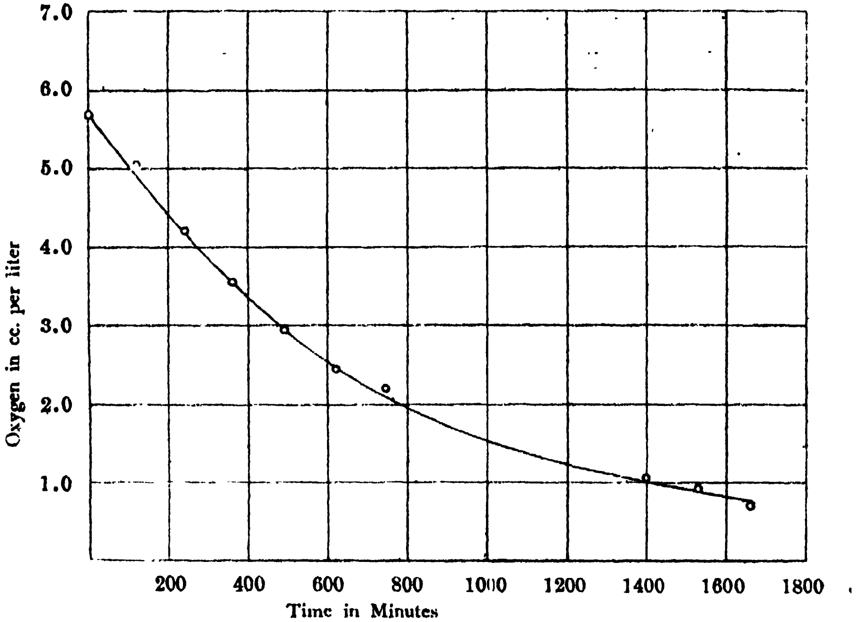


Fig. 1. The decreasing concentration of oxygen in the medium is plotted against the time. The curve thus obtained is of exponential form.

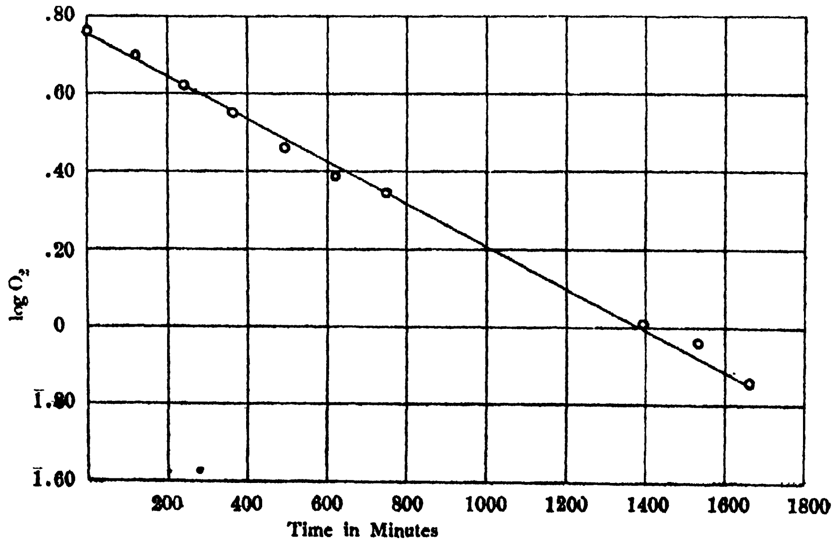


Fig. 2. Logarithms of oxygen concentration of the medium plotted against time give a straight line.

The value of k given in the table fluctuates in a somewhat wide range. It may, however, easily be regarded to lie within the limit of admissible experimental errors, if we take into consideration the fact that a wide range of variation is not infrequently met with in the determination of the respiratory rate.

GENERAL CONSIDERATION.

It has been shown that the rate of oxygen consumption in *Caudina chilensis* is proportional to the oxygen tension of the surrounding medium. This confirms the results obtained by AMBERSON and his collaborators.

As the gaseous exchange in the respiratory surface may be explained by diffusion theory alone (SCHÄFER, HALLIBURTON, BARCROFT, BAYLISS, BURNS), "secretory theory" (HALDANE) being unrequired, and the rate of diffusion of oxygen is proportional to the difference of tension of oxygen inside and outside of the respiratory surface (BURNS, p. 262 *et seq.*), the result given above would seem to render it highly probable that the rate of oxygen consumption in *Caudina* is determined by, and apparently identical with, the rate of diffusion of oxygen through the respiratory epithelium, the internal tension of oxygen being assumed to be zero or approximately so. If the internal tension of oxygen is neither zero nor approximately so, it should be diminished in proportion to the external tension, when the latter gradually decreases. Or some other factors should play a rôle to bring about the same effect as has been mentioned above. In such cases, the rate of oxygen consumption would be proportional to, but not identical with, the rate of diffusion of oxygen.

At any rate, it seems probable that the oxygen requirement of the animal is larger than the supply which diffusion can afford, and the rate of oxygen consumption is accordingly determined by the oxygen tension of the medium. The tension of oxygen, then, is the "limiting factor" (KROGH, p. 74) of the respiratory rate.

KROGH thinks "that the oxygen pressure becomes the limiting factor only when the oxygen supply fails and the oxygen tension in some of the tissues becomes zero" (p. 81). In the present material, however, oxygen tension seems to be the limiting factor even at the normal oxygen tension in the sea water.

The decrease in the rate of oxygen consumption in lower oxygen tension can not be attributed to the effect of CO_2 excreted by the animals and accumulated in the medium. Evidence in this line was secured by AMBERSON and his collaborators in various forms of animals (AMBERSON, etc., p. 176).

SUMMARY.

The rate of oxygen consumption in *Caudina chilensis* in varying oxygen tension is, at every instant, proportional to the oxygen tension of the medium at that instant.

LITERATURE.

1) AMBERSON, W. R., MAYERSON, H. S., and SCOTT, W. J. 1924. The influence of oxygen tension upon metabolic rate in invertebrates. Jour. gen. Physiol., Vol. 7, No. 1. pp. 171-176.

2) BARCROFT, J. 1925. The respiratory function of the blood. Part I. Lessons from high altitudes. London.

3) BAYLISS, Sir W. M. 1924. Principles of general physiology. 4th ed. London.

4) BURNS, D. 1921. An introduction to biophysics. London.

5) DAKIN, W. J., and CATHERINE M. G. DAKIN. 1925. The oxygen requirements of certain aquatic animals and its bearing upon the source of food supply. Brit. Jour. exp. Biol., Vol. 2, No. 3, p. 293.

6) HALDANE, J. S. 1922. Respiration. Yale Univ. Press.

7) HALLIBURTON, W. D. 1917. Handbook of physiology. 13th ed. London.

8) HENZE, M. 1910. Über den Einfluss der Sauerstoff auf den Gaswechsel einiger Meerestiere. Bioch. Zeitschr., Bd. 25, pp. 255-278.

9) *KONOPACKI, M. 1907. Über den Atmungsprozess bei Regenwürmern. Bull. d. l'Acad. de Science de Cracovie, M. n. Kl., pp. 357-431.

10) KROGH, A. 1916. The respiratory exchange of animals and man. Monographs on biochemistry. London.

11) SCHÄFER, E. A. 1898. Text-book of physiology. Vol. 1, p. 779. London.

12) *THUNBERG, L. 1905. Der Gasaustausch einiger niederer Tiere in seiner Abhängigkeit vom Sauerstoffpartialdruck. Skand. Arch. f. Physiol., Bd. 17, pp. 133-196.

13) *WINTERSTEIN, H. 1908. Beiträge zur Kenntnis der Fischatmung. Pfl. Arch. Bd. 125, p. 73.

14) *WINTERSTEIN, H. 1909. Über die Atmung der Holothurien. Arch. d. Fisiol., Vol. 7, p. 83.

15) *WINTERSTEIN, H. 1912. Handbuch der vergleichenden Physiologie. Bd. I, 2. Hälfte, p. 59. Jena.

---

* Indirectly cited.

Über die Leibeshöhlenflüssigkeit von Holothurien, *Caudina chilensis* (J. MÜLLER).

VON

KATSUMI OKAZAKI und TATSUO KOIZUMI.

In den letzten zwei Jahren haben wir das Blut und die Leibeshöhlenflüssigkeit von See-Wirbellosen inbezug auf ihren Gehalt an verschiedenen anorganischen Bestandteilen und ihre physikochemischen Eigenschaften untersucht, um eine sichere Grundlage zur Bereitung der für die physiologischen Untersuchungen dieser Tiere allgemein üblichen Salzlösung, wie der Ringerschen für den Frosch, zu gewinnen. Das Material wurde in der Nähe der marinen biologischen Station zu Asamushi gesammelt. Die Analysen selbst wurden teils in der Station, teils im biologischen Institut der Universität zu Sendai ausgeführt.

Über den Körpersaft von Echinodermen, und namentlich von Holothurioiden, sind wir noch recht ungenügend unterrichtet. Zwar ist durch die Arbeiten von FRÉDÉRICQ, BOTTAZZI und anderen[1] erwiesen, dass ihre inneren Flüssigkeiten im allgemeinen einen mit Meerwasser übereinstimmenden osmotischen Druck besitzen, über ihre quantitative Zusammensetzung jedoch liegen nur einige, allerdings dürftige Angaben vor. HENRI und LALOU[2] fanden nämlich gleiche Mengen Chlor in der Höhlenflüssigkeit von *Holothuria tubulosa* sowie von *Stichopus regalis* wie im Meerwasser. Nach BOTTAZZI[3] enthält die Leibeshöhlenflüssigkeit von *Holothuria poli* gar keine gelösten Eiweisskolloide. Auch FRÉDÉRICQ scheint die Leibeshöhlenflüssigkeit von *Holothuria tubulosa* analysiert zu haben; leider aber war seine Mitteilung uns nicht zugänglich. Inbetreff des Körpersaftes von *Caudina* konnten wir zuerst Nachfolgendes über die Ergebnisse der bisjetzt ausgeführten Untersuchung der Leibeshöhlenflüssigkeit von *Caudina Chilensis* (J. Müller) feststellen.

---

[1] F. BOTTAZZI. 1908. Osmotischer Druck und elektrische Leitfähigkeit der Flüssigkeiten der einzeligen pflanzlichen und tierischen Organismen. Ergebn. Physiol. 7., S. 246.

[2] V. HENRI et S. LALOU. 1903. Régulation osmotique du liquide interne chez les Oursins. Régulation osmotique des liquides internes chez les Holothuries. Compt. rend. société de Biolog. 55, 1224, 1244.

[3] F. BOTTAZZI. 1909. Untersuchungen über die Kolloide der Leibeshöhlenflüssigkeit und des Blutes der Seetiere. Kolloid Zeitsch. 5, S. 36.

Seit Mitsukuri[1] glaubte man bezüglich der Verbreitung dieser Spezies in Japan, dass sie auf Zenibako in Hokkaido beschränkt ist. Neuerdings wurde aber von uns gefunden, dass dieses Tier auch an der Küste von Moura bei Asamushi reichlich auftritt.

Auffallenderweise pflegt diese See-gurke sich tief in dem humusreichen Sand zu verbergen und durch seinen Schwanzteil zu atmen. Seine Behausung verrät sich, besonders zur Zeit der Ebbe, durch eine kleine Erhöhung auf schiefem See-boden, welche in der Mitte eine kleine Öffnung trägt, aufweist, und unterscheidet sich dadurch von der des *Arenicola*, mariner Anneliden, dass dieses die charakteristische bandförmige Erhöhung von Sand mit seinen Exkreten hervorruft.

Die zu untersuchenden Tiere wurden an der Küste von Moura gefangen, sofort in die marine biologische Station gebracht und solche von 130-150 mm. Körperlänge und 35-50 g. Körpergewicht ausgewählt. Die Tiere wurden oberflächlich mit destilliertem Wasser gespült, dann mit Filtrierpapier schnell abgewischt, wonach die Leibeshöhlenflüssigkeit direkt aus der Körperhöhle nach vorsichtigem Durchschneiden der Körperwandung entnommen wurde. Etwa 15 ccm. Flüssigkeit wurden in der Regel von einem Tier erhalten.

Die Flüssigkeit war weinrot und getrübt. Ihr spezifisches Gewicht betrug 1.030 bei 22° und ihr Gefrierpunkt lag, mit dem Beckmannschen Mikro-apparat gemessen, bei −1.875. Ihre Reaktion war nach Cullen und Clark Ph.=7.5–8.2 bei 22°. Spontane Gerinnung trat nicht auf.

Unter dem Mikroscope konnte man eine grosse Menge kernhaltiger Erythrocyten, ovalkonvex in Form, mit Durchmesser von 11–13 μ, und ziemlich reichlich gleichgrosse, ein und zwei Pseudopodien führende Amoebocyten und zuweilen dazu noch einige Rhomboeder von Kalkcarbonat konstatieren. Die Zahl der Erythrocyten betrug 0.15 Millionen pro kmm.

Zum Zwecke der Enteiweissung wurde die Flüssigkeit mit 3 fachen Mengen ihres Volums 20 procentiger Trichloressigsäure versetzt, gut gemischt, centrifugiert, die oben stehende klare Lösung in mehrere Portionen von bekanntem Volumen geteilt und zu folgenden Bestimmungen verwendet.

---

[1] K. Mitsukuri. 1912. Studies on Actinopodous Holothurioidea. Jour. coll. sci. imper. univ. Tokyo. Vol. XXIX, p. 251.

Von Nr. 1 bis 5: Natrium, Kalium, Kalzium und Magnesium nach KRAMMER und TISDALL[1]; Chlor nach VAN SLYKE[2]; Phosphat nach BRIGGS[3]; Sulfat nach DENIS[4]; Kohlensäure nach VAN SLYKE.

Von Nr. 6 bis Ende: Natrium nach HANS MÜLLER[5]; Kalium und Kalzium nach KRAMMER und TISDALL; Magnesium nach HAMMETT und ADAMS[6]; Chlor nach WHITEHORN[7]; Sulfat nach KLINKE[8].

Die folgende Tabelle giebt die Ergebnisse der Untersuchung wieder. Darin ist der Gehalt an einzelnen Bestandteile in Gramm pro 100 ccm. Flüssigkeit ausgedrückt, ausgenommen die Menge der Kohlensäure, die in Volumprozenten eingetragen ist.

TABELLE I.

Tier-Nr	Na	K	Ca	Mg	Cl	SO_4	HPO_4	CO_2
1	1.0420	0.0858	0.0399	0.0980	1.8650	0.2560	0.00096	4.68
2	1.0328	0.0376	0.0381	0.0982	1.9232	0.2620	0.00093	4.61
3	1.0520	0.0378	0.0387	0.0952	1.7800	0.2540	0.00087	4.27
4	1.0336	0.0360	0.0420	0.0878	1.8060	0.2560	0.00081	3.81
5	1.0454	0.0354	0.0410	0.0890	1.8250	0.2720	0.00088	4.70
6	1.0380	0.0335	0.0376	0.1100	1.8176	0.2500	—	—
7	1.0622	0.0390	0.0389	0.1162	1.8500	0.2522	—	—
8	1.0475	0.0372	0.0398	0.1092	1.8654	0.2744	…	—
9	1.0120	0.0331	0.0373	0.1023	1.8269	0.2577	—	—
10	1.0756	0.0395	0.0400	0.1157	1.8411	0.2537	—	—
11	1.0729	0.0335	0.0414	0.1085	1.7956	0.2522	—	—
12	1.0201	0.0328	0.0417	0.1082	1.8266	0.2473	—	—
13*	1.0540	0.0353	0.0400	0.1147	1.8291	0.2500	—	—
in Mittel	1.0462	0.0359	0.0359	0.1033	1.8307	0.2566	0.00089	4.43

* Mischung von aus 10 Individuen gewonnener Flüssigkeit.

---

[1] B. KRAMMER, and F. TISDALL. 1921. The direct quantitative determination of Sodium, Potassium, Calsium and Magnesium in small amount of blood. J. Biol. Chem. Vol. 48, p. 223.

[2] D. D. VAN SLYKE. 1923. The determination of Chlorides in blood and tissue. J. Biol. Chem. Vol. 58, p. 523.

[3] A. P. BRIGGS. 1922. A modification of the Bell-Doisy phosphate method. J. Biol. Chem. Vol. 53, p. 13.

[4] W. DENIS. 1921. Sulfates in blood. J. Biol. Chem. Vol. 49, p. 311.

[5] HANS MÜLLER. 1923. Eine titrimetrische Mikromethode zur Bestimmung des Natriums. Helv. chem. A. 6. S. 1152.

[6] F. S. HAMMETT, and E. T. ADAMS. 1922. A colorimetric method for the determination of small amounts of Magnesium. J. Biol. Chem. Vol. 52, p. 211.

[7] J. C. WHITEHORN. 1920. Simplified method for the determination of Chlorides in blood or plasma. J. Biol. Chem. Vol. 46, p. 449.

[8] A. KLINKE. 1924. Versuch einer massanalytischen Bestimmung des SO_4-Ions in kleinsten Mengen. Biochem. Z. 154, S. 171.

Wie oben erwähnt, betrug die Gefrierpunktserniedrigung der Leibeshöhlenflüssigkeit von *Caudina* 1.875°, während BOTTAZZI und FRÉDÉRICQ bei verschiedenen Holothurien Werte über 2.3° fanden. Dieser Unterschied beruht sicherlich auf der verschiedenen Beschaffenheit ihrer Umgebung, da *Caudina* zu den poikilosmotischen Tieren gehören soll. In Wirklichkeit waren die Proben des Meerwassers, die an einer 1.5 Meilen von Asamushi entfernten Stelle entnommen wurden, von einer niedrigeren Konzentration, wie die folgende Tabelle zeigt. Es zeigt sich deutlich der poikilosmotische Charakter von *Caudina*.

TABELLE II.

Na	K	Ca	Mg	Cl	SO_4	Δ
1.1634	0.0357	0.0431	0.1373	1.9428	0.2627	−1.885°

Inbezug auf den Gehalt an verschiedenen Mineralbestandteilen ähnelt die Höhlenflüssigkeit ebenfalls dem umgebenden Medium, enthält jedoch im grossen ganzen weniger Salze als das Meerwasser. Am deutlichsten treten Unterschiede in der Menge von Chlornatrium und Magnesium auf. Bekanntlich wird der osmotische Druck im Blut der Selachier, welche man gewöhnlich als das höchste Mitglied der poikilosmotischen Tiergruppe ansieht, durch andere Substanzen als die ihrer Umgebung bedingt, obzwar seine Höhe mit der letzteren in genauem Zusammenhang steht. Aus unseren Untersuchungen ergibt sich, dass schon bei *Caudina* ein Streben, die chemische Eigenart unabhängig von der Natur der Umgebung zu bewahren, vorkommt. Dafür spricht auch die Untersuchung von HENRI und LALOU, die die Undurchlässigkeit der Holothurien und Seeigelhaut für Salze und Rohrzucker beobachten konnten. Nach ihnen enthält ferner die Leibeshöhlenflüssigkeit von Seeigeln etwas weniger Chlor als das Meerwasser, während die von *Holothuria tubulosa* und *Stichopus regalis* einen gleichhohen Chlorgehalt aufweist wie ihre Umgebung. In dieser Beziehung steht daher *Caudina* eher den Seeigeln näher als den übrigen Holothurien.

Zum Schlusse möchten wir Herrn Professor Dr. SHINKISHI HATAI, Direktor der marinen biologischen Station zu Asamushi, unsern verbindlichsten Dank für seine vielseitige Hilfeleistung bei der Ausführung dieser Versuche aussprechen.

Some Preliminary Studies of the Influence upon Plants of the Relative Length of Day and Night.

By

YOSHIJI YOSHII.

(With four Plates)

While the question of the effects of the daily alternation of light and darkness on the growth of plants had been previously discussed, the effects of the relative length of day and night upon plants seem to have never been taken into account until in 1920, GARNER and ALLARD (4) first called attention to this subject and showed that the relative length of day and night decidedly influences the time of flowering of a plant. They found that certain plants attain the flowering and fruiting stages only when the length of a day falls within certain limits and that they become more vegetative with increased length of day (short-day plants); while other plants require a relatively long day to reach the reproductive stage and become weakly vegetative with a short day (long-day plants). This peculiar effect of the relative length of day and night on plants has been confirmed by many other investigators (3, 6, 7, 8, 9), while ADAMS in Canada (1, 2) holds a different opinion, especially as to the effect of the shortening of the daylight period on the flowering time of some plants. Although the explanation of this regulatory effect of light is as yet not given, the results obtained by GARNER and ALLARD not only present a number of interesting physiological problems but also suggest many important applications in the practical field of crop production.

In order to throw some light on this problem, I have carried out some experiments with different plants, especially with rice, of which I am now making some physiological and ecological studies. In the preliminary studies I intended to investigate whether or not GARNER'S results hold good in this part of our land, where the latitude, as well as the relative length of day and night in the vegetative season, is practically the same as that of Washington.

I. EXPERIMENTS IN 1924.

During the summer of 1924 preliminary experiments were carried on, which consisted of a series of plantings of wheat, Indian millet, buckwheat, sunflower, cosmos, morning glory, egg-plant, and soybean. Six sets of each of these plants were planted in pots. For regulating the length of day, a dark chamber was used, into which the test plants were transferred for a portion of the day; the plants received sunlight for eight hours, from eight A. M. to four P. M., or for four hours, from eight A. M. to twelve noon. One set of plants, also exposed for eight hours, was transferred daily to another chamber which was not entirely darkened. For the lengthening of the hours of illumination Mazda electric bulbs of 300 watts were used. By means of these bulbs the plants were illuminated from sunset till morning, supplementing the daylight. The greenhouse, provided with electric bulbs, was opened entirely on both sides. A set of plants (Control B), which was entirely excluded from the electric light by a black curtain, was placed beside the illuminated plants. Another set of control plants (Control A) received the natural daily period of light in the open air.

The following results were obtained: Among the plants, morning glory, the growing period of which had been known clearly from my previous paper (10)[1], showed a striking difference in the flowering dates, due to the regulating of the daylight period. The plants had germinated on June 10. Under the eight hour day and under the four hour day conditions, beginning on June 16, they came into flower on July 11 and 12 respectively. (Plate I, Fig. C). The controls exposed to the natural length of day showed the first flower on August 29. Thus the plants of short day blossomed in the early part of the summer, in only thirty-two days, when the controls had formed, as yet, no flower-bud. The plants under electrical illumination had formed no flower-bud even when the experiment ended on October 1. Therefore, the growing period was reduced remarkably by shortening the daily light period. From this evidence it is clear that morning glory is one of the most striking of the short day plants.

---

[1] This plant, planted in early summer and exposed to daylight, takes about 100 days from germination to the first flowering, but the growing period is reduced to about half of this time when it is planted in the short days in late summer or in autumn; in the greenhouse during winter the plant came into flower within [illegible] days.

All the other test plants behaved almost equally toward the light regulation. In other words, the plants of the short day type reached the flowering stage earlier than the controls, especially buckwheat, wheat, soybean, sunflower, and cosmos, which showed a marked difference. (Plate I, Fig. A and B) The vegetative development of these plants was, however, remarkably reduced. The plants exposed four hours daily grew very weakly and remained in a dwarfed form with but small flower and fruit. With the exception of sunflower and buckwheat, none of the plants under the lengthened light exposure formed a flower-bud while the controls were in blossom. Most of the controls in the open air (Control A) attained the greatest average height and weight. In the case of such plants as morning glory, egg-plant, and cosmos, however, the controls exposed to natural daylight under the glass screen (Control B) grew more strongly. Since the plants under eight hours light exposure reached earlier flowering stages and still furnished a good crop, it seems likely that a moderate shortening of the daylight period induces, for some plants, beneficial results in crop production.

The results obtained from experiments with rice plants is peculiar. In the experiments two varieties of early and late maturing types were used. While the early variety, by shortening the day length, came into head at practically the same time as the controls, the late variety showed heading almost one month earlier than the controls. (Plate I, Fig. D)

On the other hand, in order to see the effect of a long day on the growth of plants somewhat more in detail, experiments were made repeatedly in the winter of 1924 and in the spring of 1925. These experiments were done in the greenhouse. In order to have conditions as uniform as possible the plants were cultivated in an artificial nutrient solution. The species used in the experiments were mainly morning glory, buckwheat, maize, and soybean. There were two sets of each kind of plants, one set being exposed to electric light, supplementing the daylight period. The results obtained always showed a marked difference: the plants which received a long day period were, on the average, longer and heavier than the controls (Table I),[1] Buckwheat

---

[1] This is also to be noted in Plate II, Fig. A and B.

TABLE I.

(cf. Plate II, Fig. A and B).

Plant	Condition	Average height in cm.	Average weight[1] in gms.	Time of experiment
Buckwheat	Control Illuminated	41.6 64.1	17.76 21.67	April 27— May 27, 1925
Maize	Control Illuminated	53.2 87.8	40.31 67.34	"
Morning glory	Control Illuminated	18.4 30.1	26.4 39.3	June 15— July 9, 1925

[1] Three plants in a pot.

was the only plant which was in blossom at the end of the experiment on May 27 ; the first blossom of the controls opened a few day earlier than that of the illuminated plants. Morning glory formed some flower buds in the controls, while those under illumination never came into flower. The effect of long days on the growth of plants was more distinctly shown by *Salvinia*, as will be seen in Plate II, Fig. C.

Concerning this investigation, the results obtained with a shade plant will be given. In order to examine the effects of long days on a shade plant, seeds of *Chrysosplenium Grayanum* were sown in pots, and, after the plants had properly developed, one set consisting of two pots was exposed to artificial illumination during the night. At the end of the experiment they showed only a slight difference in growth, but the difference became remarkable when in autumn they were transferred to the greenhouse to continue cultivation ; the plants which had been illuminated grew rapidly, and by and by came into flower, while the controls grew weakly without the formation of flower buds. (Plate II, Fig. D) Although the explanation of this remarkable behavior of a shade plant under illumination is not at present clear, yet it is likely that this shade plant belongs to the group of short day plants.

II. EXPERIMENTS IN 1925.

Some further experiments were made in the summer of 1925, beginning on June 15. Six species of plants were used, namely, cosmos, soybean, maize, buckwheat, and rice. The results obtained in this experiment were almost the same as those in the former experiments. Therefore, an extended account of the results will be given for rice alone, only summaries being given for other species. The methods in the present experiments were essentially the same as those described in the former cases, in which the plants were transferred to a dark chamber for a portion of the day, in order to reduce the daylight period, or illuminated all night under electric lights to prolong the daylight period. Care was taken to have the conditions as uniform as possible. Some experiments were also undertaken to throw some light on the problem under discussion or to see the influence of light intensity upon the form of plant development; as well as upon the chemical constitution of a plant. For shading, a large mosquito net and a hut sheltered by reeds were used, in which one set of each of the plants was cultivated.

The results obtained in the experiments are given briefly in the following:

TABLE II.

The experiments extended from June 15 to October 5.

Plant	Condition	Date of first flower	Average height in cm.	Total dry weight in gms.
Buckwheat	Control	Aug. 24	52	63
	Illuminated	Aug. 27	58	74
	In a net	Aug. 25	66	83
	9 hour day	Aug. 15.	38	57
Soy bean	Control	Aug. 20	45	122
	Illuminated	Aug. 26	48	55
	In a net	Aug. 23	88	74
	9 hour day	Aug. 8	25	54
Morning glory	Control	Sept. 15		25
	Illuminated			18
	In a net	Aug. 4		24
	9 hour day	Aug. 2		17
Maize	Control	Sept. 5	36	17
	Illuminated	Sept. 9	44	13
	In a net	Sept. 25	54	14
	9 hour day	Sept. 3	43	16

In almost all cases the earliest flowers were obtained by the plants exposed to a shortened daylight period, including plants not tabulated here, although maize and the early maturing variety of rice headed at practically the same time as the controls. Except morning glory, the plants in the the net always came into flower later than the controls in the open air. The plants receiving longer daylight periods by means of electrical illumination reached the flowering stage later, but some plants, such as morning glory and a late variety of rice, developed into "gigantism"— in which no flower-bud is formed at all. In all cases the plants cultivated in the net grew tallest. While the plants illuminated in the night were taller than the controls, those exposed to shortened daylight periods were smaller. The greatest weight was attained by the controls — except in the case of buckwheat in which the plants grew most luxuriantly in the net. The weight of the plants in the net was greater than that of the illuminated plants, while the smallest weight was always given by the plants sheltered by the reed screeen. The plants that grew in the reduced daylight period were also less heavy. That the illuminated plants were lighter than the controls in the open air can be explained by the fact that the former were placed under the glass screen and the external conditions were not beneficial for plant growth, for the controls placed under the glass screen were more weakly developed in every case, than the lighted plants, even when they were placed side by side. This supposition is confirmed by the experiments in the greenhouse under uniform conditions, as already mentioned.

From the results obtained in these experiments and in the preliminary experiments we may draw the conclusion that:

Most of the test plants belong to the short-day plants, as GARNER and ALLARD pointed out, and they come into flower only when the length of the day falls within certain limits, and some plants continue vegetative growth under prolonged illumination, forming no flower-bud. In general, the rate of growth is proportional to the length of the daily exposure to light, but for the development of flower-buds the length of daylight required is variable according to the kind of plant under cultivation. The growth of a plant is forced by slight shading, the plants in a net screen are taller, and often heavier, than the controls in the open air, but the former generally produce flowers sparingly.

III. EXPERIMENTS WITH RICE.

Three varieties of rice differing in time of maturity were used for the series of experiments. Seedlings of rice, which were germinated in a rice field,* when about twenty centimeters high, were transplanted in pots, ten centimenters in radius and thirty centimeters high, each of which contained three plants. Fourteen pots were used for each variety and experiments were done in duplicate so that there were seven sets in all. Beginning on June 7, they were subjected to various lengths of days and to different intensities of light. One set was exposed nine hours and another five hours daily, while the third was exposed to prolonged daylight by electric illumination all night. Control B under the glass screen and Control A in the open air received full daylight. One set was shaded in a cloth net and the other in a reed screen, as already described.

The following results were obtained:

TABLE III.

Variety of rice	Control A	Illuminated	Control B	In a net	In a screen	5 hour day	9 hour day	9 hour day in half darkened chamber
				Date of first heading				
E[1]	July 29	Aug. 2	July 30	July 30	July 30	July 29	July 29	July 30
L	Aug. 22			Aug. 9		Aug. 4	Aug. 4	Aug. 13
VL	Aug. 26		Aug. 27	Aug. 19	Not raised	Aug. 4	Aug. 4	Aug. 18
				Average dry weight in gms. (excluding the root and the ear)				
E	10.8	8.6	7.8	11.8	8.7	8.1	11.3	10.8
L	22.2	17.0		18.8		12.7	15.1	15.2
VL	15.1	16.1	10.1	17.6	4.2	9.8	12.1	12.8
				Average dry weight of root in dgms.				
E	4.6	1.6	1.8	2.7	0.4	1.8	4.4	2.6
L	9.5	3.8		4.4		3.2	3.8	4.5
VL	5.1	3.3	2.8	4.3	0.7	3.3	4.6	3.4

---

* The writer expresses his hearty gratitude to Mr. Y. TERASAWA, in the Experimental Agricultural Station of Miyagi Prefecture, who kindly supplied him with seedlings of rice.

Variety of rice	Control A	Illuminated	Control B	In a net	In a screen	5 hour day	9 hour day	9 hour day in half darkened chamber
				Average height in cm.				
E	82	89	74	98	80	105	92	86
L	101	109		110		73	75	90
VL	85	96	74	97	81	71	79	76
				Average number of basal branches (The numbers in brackets denote secondary branches)				
E	10.7 (3.7)	11.0 (1.0)	9.3 (2.3)	11.3 (3.7)	5.0 (5.7)	11.3 (1.0)	11.7 (0.7)	11.7 (1.0)
L	9.7 (1.3)	8.7		8.7 (0.7)		9.7 (6.3)	13.0 (3.3)	13.0 (0.3)
VL	10.0 (0.3)	9.7	7.3 (2.0)	12.3	2.7 (0.7)	12.3 0.7)	14.3 (3.0)	13.7
				Average number of ears				
E	10.0	8.3	5.3	10.7	5.0	10.0	10.4	11.2
L	4.8	None		7.8		6.7	12.5	12.8
VL	8.5	None	5.7	10.3	2.7	9.8	14.0	13.5
				Average length of ears in cm.				
E	18.5	17.5	15.0	16.0	14.0	15.0	17.0	18.3
L	16.5	None		18.5		17.0	16.3	15.3
VL	18.5	None	17.5	16.0	15.0	15.5	15.0	13.5
				Average number of grains in one ear				
E	(60)	(40)	32	(50)	(35)	(45)	(40)	(70)
L	78	None		(70)		(40)	75	95
VL	(80)	None	50	(60)	81	53	46	97

1) E, Early variety; L, Late variety; VL, Very late variety.

THE TIME OF HEADING.

Under various conditions of light the early variety came into ear almost at the same time, the only exception being the set exposed to electrical illumination, which came into the first heading in the beginning of August, a few days later than the controls. Thus plants of long day blossomed practically at the same time as the controls exposed to natural day-lengths. Concerning the date of flowering of the early variety, therefore, it seems that the effect of reducing the light period by shortening the exposed daylight period was the same as that of reducing the intensity of light by shading in the net or in the reed screen.

Although the effect of the short daylight period is not so marked in the late variety, as in the case obtained in the previous experiments in 1924, yet the heading came much earlier than the controls; while the latter came into the first heading on August 22, the plants of a nine and of a five hour day had ears eighteen days earlier. The plants in the net also showed a somewhat earlier heading. The difference was more marked by the very late variety. A remarkable phenomenon in the experiments is that in both late varieties of rice under a lengthened daily period by electrical illumination, vegetative development continued and heading did not occur at all. Thus they experienced the phenomenon described by GARNER as "gigantism." The late variety of rice is therefore apparently a short-day plant, while the early variety is indifferent to the regulation of the light period.

WEIGHT.

The early variety of plants which received nine hours of light daily attained greater weight than those exposed to lengthened illumination. The reverse is the case with the late and the very late varieties. The shaded plants in the net always reached greater weight. In the late varieties, the controls exposed to the full daylight period (Control A) always showed greater weight than those of the short daytime. Hence, the rate of growth, as far as the weight in concerned, is directly according to the length of day, as pointed out by GARNER. ADAMS, and DEATE. Under a five hour light exposure, the weight of the plants was the smallest, except in the case of those plants cultivated in the reed screen. From these results we come to the conclusion that light affects the different varieties of rice differently,—while the early variety attained great weight by reduced illumination (provided that it does not go under the minimum limit) the late and very late varieties grow rapidly by prolonged exposure. This is related to the fact that these late varieties, when receiving full daylight supplemented by electric illumination during the night, never come to flower. The cause of the great weight of the shade plants in the net may be sought as other than the effect of the relative length of day and night.

The period of light exposure does not as markedly affect the weight of the root. The greatest growth of the root was always attained by the controls exposed to full daylight (Control A), and the difference in the varieties was, in this case, not very conspicuous.

HEIGHT.

The average height of plants, measured to the top of the leaf, differs in the different varieties. In the early variety the plants exposed to light only five hours daily were the tallest, the next being the shaded plants in the net and the plants exposed nine hours daily; while the controls remained rather smaller. The late and the very late varieties attained almost the same height. Just as in the case of weight, the average height of the plants in the net and those illuminated by electric light were the greatest. The plants under the glass screen — the controls of the illuminated plants (Control B) — always showed very slight results both in weight and height, irrespective of the difference in the varieties. Taking this into account, it may be concluded that the growth of the late varieties would be taller and heavier if they had been cultivated in the open air, and at the same time illuminated by the electric light during the night.

STOOLING.

The number of basal branches is important for crop production, as it directly concerns the number of ears of a plant. The late and very late varieties behave almost identically in stooling under the influence of illumination. The greatest number of branches was attained by the late varieties in the plants of short day under nine hours of light exposure both in dark and half dark chambers, and even under five hour exposure an increase of stooling took place, while but very little branching was given by the electrically illuminated plants. On the other hand, in the early variety a marked increase in the number of branches was shown when under electrical illumination in the night or when shaded in the net, although an increase of stooling was also shown by the short-day plant.

EARS.

The number of ears naturally differs in accordance with stooling.

As to the number of ears, similar results were shown by the early and the late varieties, which behave almost contrary to each other in other respects. The greatest number of ears was always a result of the moderately shortened day period; viz., under the nine hour light exposure out of the dark, as well as the half dark chambers. The shaded plants in the net also increased in the number of ears and even in the weak illumination of five hours daily a slight increase in the number of ears took place. The plants under glass screens (Control B) as well as in the reed screen alway showed only a slight number of ears. It appears in the last case that the decreased light intensity caused very weak growth in the plant. From the results obtained it may be concluded that the increasing of ears comes from decreasing light intensity, either by the shortening of natural day periods or by shading with a cloth net. The longest ear was attained by the control plants exposed to full daylight periods (Control A), but the difference in length under various conditions of illumination was not striking.

NUMBER OF GRAINS.

To my regret, most of the grains of the test plants were eaten by sparrows before the end of the experiments and the total number of grains of one ear is not given. From the small number of ears not injured, it is supposed that the largest number of grains of one ear was also attained by the moderately short day plant.

The general conclusions to be drawn from the experiments with the varieties of rice is that the early and the late or the very late varieities of rice respond to light quite differently, not only in the time of flowering, but also in the rate of growth. As to the time of flowering, the early variety is almost indifferent to the influence of light, while the late varieties respond to it markedly and come into flower by the short day, but no form of flower-bud is obtained by the long day. The late varieties grow strongly under prolonged exposure, with an increase in branching under reduced daylight period, while the early variety attains great weight under a shortened day length, and branches a great deal under prolonged day length.

IV. DISCUSSION.

On the basis of their experiments, GARNER and ALLARD (4) grouped the plants into two categories, long day and short day plants, depending upon the photoperiod or length of daily illumination which results in the production of flowers. The results obtained in my experiments with several kinds of plants showed that most of them belong to the short day plants, but such plants as the early variety of rice and Indian millet proved to be neither short nor long day plants, since they came into flowering in long days as well as in short days at practically the same length of time These results and the analysis supporting evidence by ADAMS' experiment (2) lead us to a tentative conclusion that there is probably another group of plants which are nearly or entirely indifferent to photoperiods, but nevertheless are affected by other factors as to the time of flowering. For these plants no critical photoperiod is essential to the initiation of the flowering stage, but they can flower at any length of daylight, provided that light is sufficient to permit the growth of the plant.

One more important result obtained in this experiment is that the early and the late varieties of rice respond to light regulation quite differently. The late-maturing variety of rice is one which flowers and fruits under the influence of the short day. This would be the main reason why the late variety comes into heading only in the late summer. A fundamental distinction rests on the difference in their susceptibility to the reduction of the day length in the late summer or autumn. It is interesting to compare this property of the late variety of rice with that of the spring types of wheat and other cereals, which, according to GARNER and ALLARD (5, p. 877), respond to the increasing day length of spring. Thus, the late varieties of rice are forced to flower and fruit by reducing the daylight period, but not by the prolongation of daylight periods by electrical illumination. This result is important in the breeding of these varieties of rice.

The curious behavior of winter wheat as to growth and distribution has long been known without being explained, but WANSER (8) found different responses to the photoperiod in different varieties of wheat (p. 314) He concluded that the photoperiod is the key to the distinction between winter and spring wheats. GARNER and ALLARD (5) also called attention to this point. Moreover, circumstancial evidence is obtained by them which already indicates that the varieties of a plant offer many interesting problems for discussion. Among them the varieties of soybean respond to daylight periods quite differently (4), the very late varieties (Tokyo and Biloxi) never blossomed under illumination of more than thirteen hours daily. Another remarkable result is that stooling in the spring types of wheat, rye, and barley was greatly increased under the relatively short, natural day length of winter, as compared with the artificially lengthened light period. This condition is reversed in the winter wheat and rye. (p. 899)

OAKLEY and WESTOVER (6) have found that the seedlings of various varieties of alfalfa respond to the photoperiod so differently that this property can be adopted for their quick identification. The results obtained recently by TINKER (7) with the varieties of timothy (*Phleum pratense*) and other plants has confirmed the above statement. From the results obtained by these investigators with cereals and other plants, as well as those which I found in experiments with rice, the conclusion may be drawn that photoperiodism is a critical factor for the distinction of the varieties of a certain plant. Hence, I should like to say that the most interesting and important results may be obtained by studying the photoperiod of varieties of various useful plants, thus securing perhaps, an important application in crop production and plant breeding.

It is one of the important problems of plant breeding to secure varieties of various useful plants adapted to different seasons of the year in any given region. Since it is known that the different behavior of early and late, as well as spring and winter, maturing varieties of some plants should be attributed to response to the relative length of day and night, therefore, any detailed studies along this line will give very important results for the plant breeders.

V. SUMMARY.

1) Most of the test plants belong to the short-day plants and they come into flower only when the length of the day falls within certain limits.

2) There is, however, another group of plants which are nearly or entirely indifferent to photoperiod, but are affected for the time of

flowering by other factors.

3) The rate of growth is proportional to the length of daily exposure to light. The growth of a plant is also forced by slight shading but in this case the plant generally produces flowers sparingly.

4) The early and the late varieties of rice respond to light regulation quite differently not only in the time of flowering but also in the rate of growth; the late variety is a short day plant, while the early variety is indifferent to the regulation of the light period.

5) Photoperiodism is a critical factor for the distinction of the varieties of a certain plant and most interesting and important results may be obtained by studying the photoperiodism of varieties of various useful plants.

LITERATURE CITED.

1) ADAMS, J., 1923. The effect on certain plants of altering the daily period of light. Ann. Botany, Vol. XXXVII, p. 75.

2) ADAMS, J., 1925. Some further experiments on the relation of light to growth. Amer. Jour. Bot. Vol. XII, p. 398.

3) DEATE, M. E., 1925. The effect on plants of the increase and decrease of the period of illumination over that of the normal day period. Amer. Jour. Bot. Vol. XII, p. 384.

4) GARNER, W. W., and ALLARD, H. A., 1920. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. Vol. XVIII, p. 553.

5) GARNER, W. W., and ALLARD, H. A., 1923. Further studies in photoperiodism. Jour. Agr. Res. Vol. XXIII, p. 871.

6) OAKLEY, R. A., and WESTOVER, H. L., 1921. Effect of the length of day on seedlings of alfalfa varieties and the possibility of utilizing this as a practical means of identification. Jour. Agr. Res. Vol. XXI, p. 599.

7) TINCKER, M. A. H., 1925. The effect of length of day upon the growth and reproduction of some economic plants. Ann. Botany, Vol. XXXIX, p. 721.

8) WANSER, H. M., 1922. Photoperiodism of wheat; a determining factor in acclimatization. Science, n. s., Vol. LVI, p. 313.

9) WANN, F. B., 1925. Some of the factors involved in the sexual reproduction of *Marchantia polymorpha*. Amer. Jour. Bot. Vol. XII, p. 307.

10) YOSHII, Y., 1926, Über die Reifungsvorgänge des *Pharbitis*-Samens mit besonderer Rücksicht auf die Keimungsfähigkeit des unreifen Samens. Jour. of the Faculty of Science, Imper. Univ. of Tokyo, Sec. III, Botany, Vol. I, p. 1.

Explanation of Plates.

PLATE I.

A, Cosmos; B, Buckwheat; C, Morning glory.

Photographed July 14, 1924.

The plants at the left were exposed to full natural daylight, while the plants at the center received daylight eight hours daily and those at the right four hours daily.

D, Late variety of rice.

Photographed August 28, 1924, when first heading appeared on the plants at the left which were exposed to the full daylight. The plants at the right received eight hours daylight daily from June 10. They came into first heading on July 30.

PLATE II.

A, Buckwheat; B, Maize; C, *Salvinia natans.*

The plants were cultivated in Knop's nutrient solution in a greenhouse from April 27 to May 27, 1925. The plants at the left were exposed to a full daylight period, supplemented with electrical illumination in the night, while those at right are controls.

D, *Chrysoslenium Grayanum.*

Photographed October 10. The plants at the left were exposed to the daylight plus electrical illumination in the night, those at the right were exposed to daylight only.

PLATE III.

Late variety of rice.

Photographed September 1, 1925.

The plants were subjected to various lengths of day and to different light intensities.

A, Plants sheltered in a net. B, Control plants, exposed to full natural daylight. C, Plants exposed to the electrical illumination in the night, in addition to natural daylight. D, Plants exposed to daylight nine hours daily. E, Plants exposed to daylight five hours daily. F, Control plants.

PLATE IV.

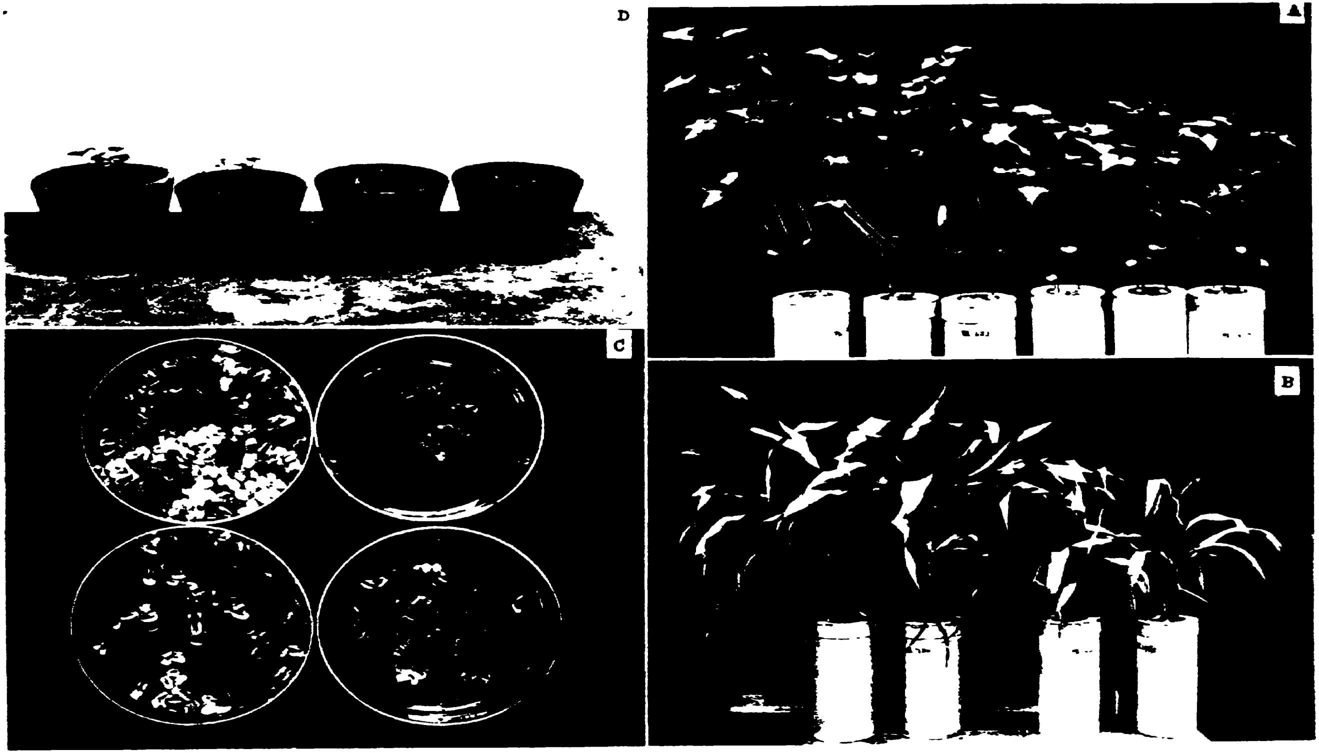
Three varieties of rice at the end of the experiments (Photographed October 1).

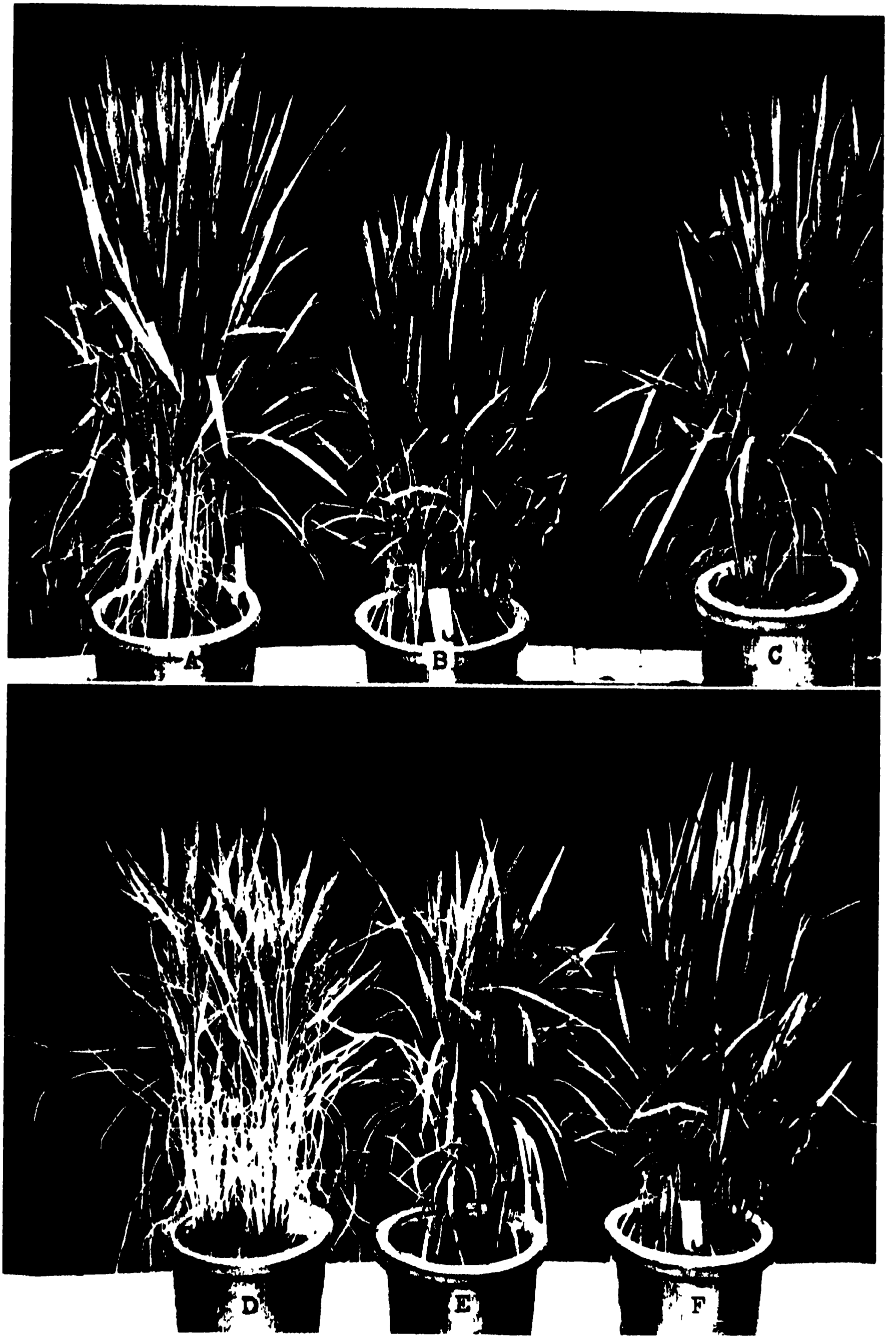
Fig. 1, Early variety. Fig. 2, Late variety. Fig. 3, Very late variety. A, The plants of nine hour in a half darkened room; B, Plants exposed to electrical illumination in the night, supplementing the natural daylight. No heading is formed by late and very late varieties. C, Controls exposed to the natural daylight period. D, Plants of nine hour day. (The grains of rice were eaten by sparrows).

A
B
C
D

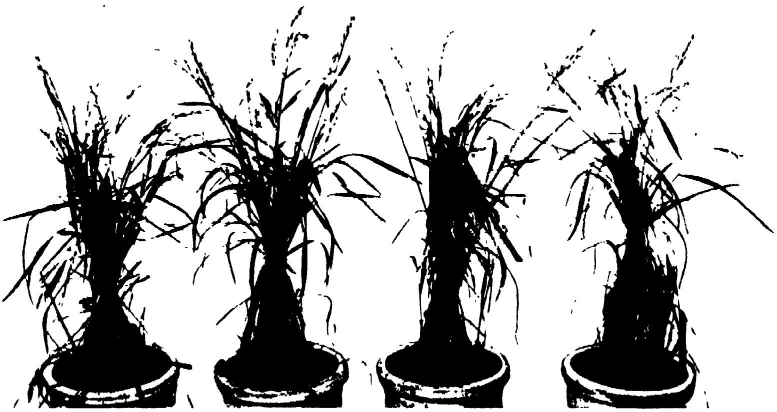
Y. Yoshii

Plate II.

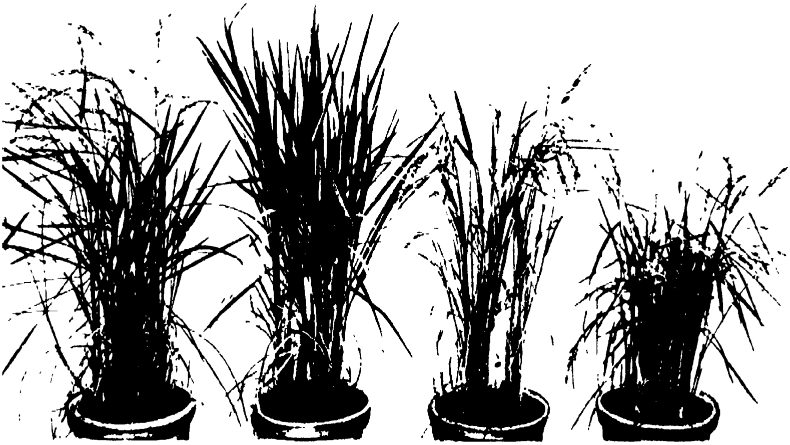



A
B
C
D
E
F

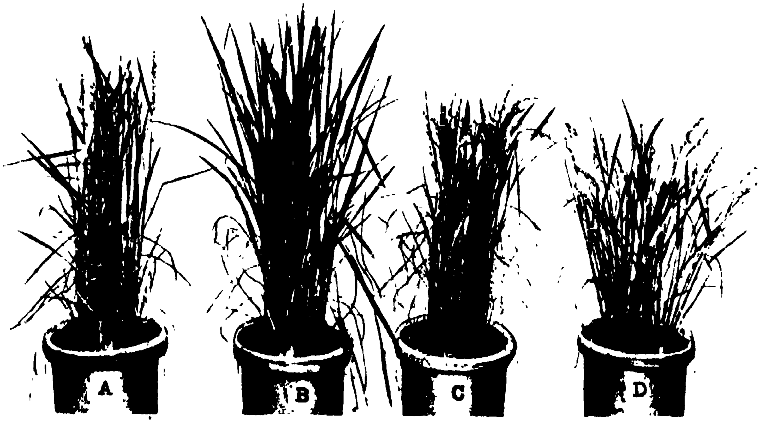
I



2



3



Grossenverhältnis der Geschlechtschromosomen von *Rumex Acetosa*, L.

VON

TOMOWO ONO.

Seit 1923 ist unsere Kenntnis der Geschlechtschromosomen der Pflanzen sprungweise gestiegen. Jetzt können wir bereits 26 Beispiele davon aufzählen. Die Pflanzen der Beispiele verteilen sich auf 14 Gattungen und 10 ganz verschiedene Familien; die Beispiele im Tierreich sind gegenwärtig ja schon unzählbar geworden. Unter diesen zahlreichen Beispielen stehen aber die Fälle von *Rumex Acetosa*[1] und *R. thyrsiflorus*[2] ganz vereinzelt, nicht nur im Pflanzenreich sondern auch im Tierreich. Bei der heterotypischen Kernteilung dieser Pflanzen tritt ein eigentümliches dreiteiliges Chromosom hervor, welches aus einem X-Chromosom und zwei Y-Chromosomen besteht. Beim *Protenor*-Typus gibt es kein Y-Chromosom. Beim *Lygaeus*-Typus stehen ein (nicht mehr) Y-Chromosom und ein oder mehrere X-Chromosomen gegenüber, und Y ist immer kleiner als X. Bei *Rumex Acetosa* und *R. thyrsiflorus* kommen aber zwei Y-Chromosomen vor, und zwar sieht die Gesamtmenge der Y-Chromosomen merkwürdigerweise grösser als das X-Chromosom aus. Bevor wir auf dieses Problem noch näher eingehen. ist es nötig zu entscheiden, ob die Gesamtmenge von den zwei Y-Chromosomen wirklich grösser als das X-Chromosom ist. *Rumex Acetosa* ist ein gemeines Unkraut in der Nähe von Sendai. Unten möchte ich die numerischen Werte der Grösse von X- und Y-Chromosomen dieser Pflanze angeben.

Das Material wurde mit CARNOYS Lösung fixiert und die Schnitte wurden mit HEIDENHAINS Eisenhämatoxylin gefärbt. Für die Messung der Chromosomen wurden bisjetzt verschiedene Methoden von den verschiedenen Forschern benutzt (Siehe! TISCHLER, 1922, S. 621–22).

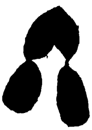
---

[1] KIHARA, N. und ONO, T. 1925. The sex-chromosome of *Rumex Acetosa*. Zeit. Abst. Vererb. Bd. 39, S. 1–7.

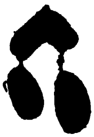
[2] MEURMAN, O. The chromosome behaviour of some dioecious plants etc. Soc. Scient. Fenn., Comm. Biol. II, 3. S. 1–104.

Alle diese Methoden sind aber für die genaue Messung der Chromosomengrösse nicht geeignet. Zur vorliegenden Untersuchung wurde darum die folgende Methode benutzt. Zuerst wurden die Chromosomen mit Hilfe des ZEISSchen Zeichenapparates möglichst genau ca. 3500 mal vergrössert auf Papier gezeichnet (Textfig. 1-5). Diese Bilder wurden dann wieder durch den LEITZschen Projektionsapparat 17 mal vergrössert. Der Flächeninhalt der einzelnen Chromosomen wurde daraufhin mit dem Planimeter gemessen. Zur Vergrösserung wählte ich immer die Seitenansicht der heterotypischen Meta- oder Anaphase in der Pollenmutterzelle. In diesem Stadium erfährt das X-Chromosom eine Krümmung, zur Folge mit zwei hängenden Armen von ungleicher Länge. Die zwei Chromosomen (y_1 und y_2) binden sich je eins an die Enden der Arme. Zwischen den beiden Y-Chromosomen ist immer ein kleiner Unterschied bemerkbar. Das grössere wird y_1 und das kleinere y_2 genannt werden. Es ist immer die Regel, dass das y_1 am Ende des kurzen, das y_2 am Ende des langen Armes des X-Chromosomes sich orientieren und dass vom X-Chromosom das y_1 stets etwas früher als das y_2 nach dem Pole sich zu trennen pflegt. Die Messungsergebnisse sind in der folgenden Tabelle zusammengestellt.

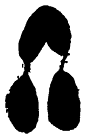
1



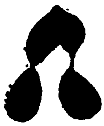
2



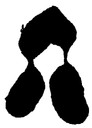
3



4



5



	X	y_1	y_2	y_1+y_2
Textfig. 1	106.4 cm²	74.8 cm²	70.6 cm²	145.4 cm²
„ 2	102.7	86.1	68.4	154.5
„ 3	101.9	77.3	67.9	145.2
„ 4	107.3	73.0	68.9	141.9
„ 5	94.2	82.7	64.3	147.0
Durchschnitt	105.5	78.8	68.0	146.8

Aus dem obigen Resultat kann man natürlich den Schluss ziehen, dass bei dieser Pflanze das X-Chromosom kleiner als die Y ($=y_1+y_2$) Chromosomen ist. Das Grössenverhältnis von X und Y ist etwa 100 : 139.

Yeasts Isolated from Flower Nectar.

BY

TADAO JIMBO.

(With six Tables and two Plates)

I. INTRODUCTION.

Yeasts found in flower nectar, so-called "nectar-yeasts," have been investigated by REUKAUF (1), STOLTZ (2), HILKENBACH (3), SCHUSTER and ULEHLA (4), SCHOELLHORN (5), and HAUTMANN (6).

REUKAUF especially treated a yeast found in the nectar of *Salvia pratensis*, *S. verticillata*, etc., and advanced a view that generally each kind of flower lodges specific kinds of yeasts on account of the different qualities of the nectar.

STOLTZ investigated yeasts from twelve kinds of flowers in Germany. He often found so-called "Kreuzform," namely cruciform cell-colonies, and recognized a relationship between insects visiting flowers and the occurrence of yeasts in the flowers; he never found yeasts in closed buds.

HILKENBACH made an extensive research on the distribution of yeasts on various parts of plant bodies, especially stigmas, and described twelve forms of yeasts which he had isolated. He investigated the stigmas of about ten flowers in each of about one hundred species of plants in the botanical garden of Kiel, Germany, in the summer of 1910 and in the spring of 1911, making a culture from each flower, and found yeasts in 60% of the flowers of most of the plants in the summer and in 20% in the spring. He also very often found yeasts in spurs, on pollens, on floral leaves, and even on the leaves of *Drosera*, etc.

Clear evidence that the infection is effected for the most part by insects, although the wind also plays some part in it, is shown by the facts that there was less infection in flowers pollinated by the wind than in those pollinated by insects, that closed buds were quite sterile, that flowers covered with gauze to keep out insects showed a percentage of infection as small as in anemophilous plants, that the rate of infection increased according to the increase of the temperature, i. e., no infection in the early spring and the highest rate of infection in the summer, and that yeasts were found only seldom in plants in rooms and in the greenhouse. The author could very often isolate from the bodies of various insects the same yeasts as were found in the flowers.

This author distinguished twelve forms of yeasts among his cultures. He did not recognize specific relations between the form of yeast and the kinds of flowers in which it was found and observed that most of the forms were found in nearly all kinds of flowers. He described the morphology of those cultivated on " Biomalz " with agar. Among the twelve forms, three forms are white, two grey-white, two pink, one yellow, two yellow-brown, one grey-brown, and one violet, one of the pink forms being identical with *Torula glutinis* PRINGSHEIM et BILEWSKY (7). One of the white forms was found to be in " Kreuzform " in the nectar of *Aquilegia* and on the stigma of *Digitalis purpurea*. Ascospore formation was never found. On agar media, the yeasts show strong growth on 10% glucose or maltose, medium growth on saccharose, and weak growth on lactose, without the addition of minerals. The optimum temperature for growth is 16–18°C. Light retards their growth.

SCHUSTER and ULEHLA investigated about sixty species of plants in Prague in the spring and summer of 1912. They found infection in twenty-three species, from which they isolated ten forms of yeasts. Two of them were rather widely distributed in various plants, while the other white, red, brown, and violet forms were each found in a single species of plant. One of these two forms showed " Kreuzform " in the nectar, as well as in some culture media, and was recognized to be identical with that mentioned by STOLTZ. These investigators noticed that the infection is mostly brought about by insects visiting the flowers, depending on weather conditions.

SCHOELLHORN made investigations on nectar-yeasts from winter plants. He isolated from the nectar of *Salvia pratensis* a kind of yeast, *Nectaromyces cruciatus* SCHOELLHORN, which often formed " Kreuzform," especially in a culture medium which contains 20% glucose, 15% saccharose, 1% mannite, 3% K_2SO_4, 1% K_2HPO_4. Besides this, he isolated ten forms of *Torula* from other plants. Frequently they also showed "Kreuzform" formation, for which honey served as a favourite medium. These yeasts never formed ascospores. He recognized that as a general rule the same kinds of flowers blooming in different places lodge the same kinds of yeasts at the same time every year, and ascribed the greater part of infection to the bee. He found less infection in alpine plants than in plants of the plains.

HAUTMANN, who treated a kind of nectar-yeast, *Anthomyces Reukaufii*, also paid much attention to the "Kreuzform." He came to the conclusion that a low concentration of nitrogen (less than 0.001%), a high concentration of sugar (more than 20% for saccharose), and good ventilation are necessary for "Kreuzform" formation.

Summarizing the works of these authors, we find the main points of our knowledge about nectar-yeasts up to the present may be stated as follows: 1) Yeasts are very frequently found on various plants, in the flower nectar, as well as on the stigmas, other parts of the flowers, and other organs, 2) they are distributed mainly by insects, the wind playing a lesser part, consequently, a cold climate and rainy weather hinder distribution, 3) some authors maintain that a definite plant lodges definite yeasts, but others deny this, 4) about ten (at most twelve) forms, which are white, red, yellow, brown, or violet, were isolated by the investigators, 5) ascospore formation was never observed, 6) the nectar-yeasts very often assume in nectar a peculiar form of cell colonies, so-called "Kreuzform," which is perhaps especially brought about by a high concentration of sugar in the nectar.

These investigations on nectar-yeasts were all carried out in Europe. No research along this line has been made in Japan until now. Following the suggestion of Professor Dr. HANS MOLISCH, I have engaged in this work in Sendai since the autumn of 1924, the greater part of the experiment has been done since April 1925, during the course of my third year as a student of the Biological Institute of the Tohoku Imperial University.

I wish to express to Professor H. MOLISCH, Professor Y. YOSHII, and Professor S. HIBINO my appreciation and thanks for their valuable suggestions and advice, as well as to Dr. T. ITO for the verification of the names of the phanerogams which I investigated.

II. EXPERIMENTS.

During April, May, and June 1925, 273 flowers of twenty-three species of plants blooming in the city of Sendai and its neighbourhood were examined with regard to yeast infection of their nectar, and all the forms of yeasts thereby found were isolated and their principal characters investigated.

1. *Methods.*

Each flower collected was cut off *in situ* with sterilized scissors, and was at once put into a glass tube containing a piece of wet cotton at the bottom and closed with a cotton stopper, the whole of which was sterilized beforehand. The collection was then carried to the laboratory. When the flower was too large to put into the tube, as in the case of *Camellia*, it was wrapped in sterilized paper.

The greatest part of the nectar was taken out by means of a sterilized, pointed, thin glass tube, the canal of which was stopped with a piece of cotton near the upper end to prevent infection by the breath, and was blown into a melted culture medium in a test tube, and the mixture being then poured into a Petri dish.

The culture medium was composed of the following:

Knop's solution	100 cc.
Saccharose	10 gms.
Agar	1.8 gms.

The formula for Knop's solution used here was as follows:

Distilled water	1000 cc.
Calcium nitrate	1.0 gms.
Potassium nitrate	0.25 gms.
Potassium biphosphate	0.25 gms.
Magnesium sulphate	0.25 gms.
Iron trichloride	trace

The Petri dish was kept under a bell jar on the table in the laboratory.

The culture media in the Petri dishes inoculated with the nectar sometimes remained sterile.

This proves that the nectar had been sterile, as far as organisms which are adapted to this medium are concerned. Generally, however, various colonies, not only of yeasts but of other fungi or of bacteria, appeared in a dish, often in great numbers. Although under ordinary circumstances the colonies of other fungi and bacteria were easily distinguished from yeasts by the naked eye or under a microscope, owing to their morphological characters, it was often not so easy to identify the colonies of the same kinds of yeasts among many yeast colonies. In this case, the appearance of the colony and the form and size of the cell were the only available criteria in identification, and direct observation of the colonies under the microscope was the only way of observing such features of the cell, for it was quite impossible to make a pure culture from each of the many colonies and to compare every character or even to investigate the cells closely in microscopical preparations taken from each. In consequence, the identification might not be an accurate one, nevertheless, it was regretfully inevitable.

Pure cultures of all the forms of yeasts in each Petri dish were prepared and kept in streak- and stab-cultures for further identification with those from other dishes, and later the collection of such pure cultures was investigated to identify the same forms among them. At this time manifold morphological characters of the colony and the cell (in microscopical preparations) in the plate-culture and the appearance of the streak- and stab-cultures were taken as the criteria.

Thus I have got a table on the distribution of these nectar yeasts, Table II, and the sample of every form of yeast, which was closely investigated concerning its principal characters which are described in the following section.

2. *Distribution of the yeasts.*

Twenty-three species of plants, as are cited in Table I, were investigated with regard to the nectar of their 273 flowers which were blooming from April 20 to June 27 of the year 1925 at five places in the city of Sendai and its neighbourhood, namely: A) the garden of the Biological Institute of the Imperial University in Sendai, situated in the city, B) a garden in the city, which is about one kilometer from the former, C) a woody terrace, called Mukō-yama, in the south-western part of the city beyond a river, D) the environment of a hill, named Taihaku-san and about six kilometers west of the city, E) Mt. Zaō, 1840 meters in altitude and about fifty kilometers distant, south-west of the city of Sendai. I will call these five places briefly A, B, C, D, and E, respectively.

Less than ten flowers of a single species of plant in a single place were investigated at a time, but often the same species was investigated repeatedly in different places or on different days or both in different places and at different times. In any case, older flowers, not those which seemed to have just opened, were chosen. The flowers were always collected after at least one fine day, never just after rain. These precautions are necessary to get average data concerning the distribution of the yeasts.

Almost all of the twenty-three species, and 120 (44%) among 273 of the flowers, lodged yeasts, which belong to twenty-two forms, ten forms of them being scarce.

Although sometimes the nectar was quite free from organisms, it was usually infected with diverse numbers of bacteria, yeasts, or other fungi, any of them alone, any two, or all together in a single flower. In the present investigation I have paid no further attention to bacteria and fungi other than yeasts.

The data along this line are put together in Table II. Statistical considerations based upon these data and made from various points of view are shown in Tables III, IV, and V. According to these tables I may conclude as follows:

1. The nectar-yeasts are widely distributed in nature, not only in the town and in the field but also high on the mountain.

2. The same forms of yeasts are found in the nectar of various plants and in different places.

3. The some species of plant very frequently shows different rates of yeast-infection and different forms of yeasts according to the place and the time, in other words, no definite relation between the species of plant and both the rate of infection and the form of yeast can be recognized.

4. The rate of infection increases with the seasonal rise in temperature, as some of the preceding authors have pointed out.

3. Description of the yeasts isolated.

The principal morphological characters of the twenty-two forms of nectar-yeasts which I isolated are given in Table VI.

The conditions of culture in which the yeasts show such characters are as follows:

1. The composition of the culture medium is the same as above described.
2. Temperature about 25°C.
3. In darkness.

The plate-cultures were observed for two weeks after inoculation, and consequently the descriptions under "Plate-culture" refer to the characters of the colonies and the cells during that time, those of older cultures being outside our attention here. The properties of "Colony" are concerned with the colonies on the surface of the medium, except that the peculiar properties of the edge of "Deep-seated colony," or colony within the medium, are particularly mentioned. The size of the cells was measured in the mother cells of cell colonies, but in a few cases, in which cell colonies were so scarce, I had to measure singular cells. The latter cases are especially noted in Table VI. The slime-halo was observed in fine Chinese ink.

The streak-cultures and the stab-cultures were observed for one month. Of course the colonies of the streak-cultures and the stab-cultures generally show properties similar to those of the plate-cultures, e. g., similar colour, similar lustre, etc., therefore I shall describe only those points which differ from the plate-cultures or are particularly remarkable.

The slime-halo is lacking in young cells in any case.

"Oil-drop" means inclusions of the cells, which appear as minute granules or drops of diverse sizes and are stained orange-red by Sudan III. In young cells the oil-drops are so small that they look like mere granules, but in most of the forms of yeasts they grow larger according to the growth of the cells and give rise to yellowish refractive drops.

Vacuoles are often conspicuous and are often large in most of the forms. They are of less importance as a specific character of each form.

By the repetition of a culture a few times under the same conditions, it was proved that every character described here holds constantly in each form of yeast.

Ascospore formation has never been observed in any form, in spite of the culture being on gypsum blocks with bottoms immersed in water at about 25°C.

Fermentation tests, with Smith's fermentation tubes filled with Knop's solution containing 10% sugar and kept at about 25°C, showed negative results with dextrose, saccharose, maltose, and lactose. These yeasts apparently have no ability for alcoholic fermentation with these sugars.

"Kreuzform," which has never appeared in the cultures, was found only in a few cases in the nectar, although I examined the nectar of most of the flowers directly on inoculation. In two flowers of *Iris sanguinea*, collected in B-place on June 11, the "Kreuzform" was found among normal cells in the nectar, and the nectar of one flower of *Lonicera grac.*, collected in A-place on May 13, showed a great number of cells which were in "Kreuzform." It was proved by the cultures that in the first two flowers all the cells, both in the normal forms and in the "Kreuzform," were of the yeast No. 13 and in the other all of the cells, which were assuming "Kreuzform," were of No. 12. Moreover it was peculiar that, at the same time, the other flowers of *Iris* and *Lonicera* which were also proved to contain the yeasts No. 13 and No. 12, respectively, did not show the "Kreuzform." According to these facts we may believe that the "Kreuzform" is a modification of the form of the yeast cells sometimes caused, perhaps, by some conditions of the nectar, and that the yeasts No. 12 and No. 13 can assume the "Kreuzform."

4. Extracellular-oil formation of form No. 19.

Form No. 19 is peculiar among the twenty-two forms by its specific character of forming oil not only in the cell but also out of the cell. In the plate-culture, in the way above mentioned, a great number of oil drops appear among the yeast cells in the colonies.

Young colonies, which are only about three days old, are proved by microscopical preparations to have already formed extracellular oil drops, although the drops are too small to be observed directly in the colonies under the small magnification of the microscope. In about five day old colonies abundant small drops can be observed directly, and in about seven day old colonies they become quite numerous and larger, being scattered all over the colonies. Colonies at the bottom of the dishes also show extracellular oil drops.

The extracellular oil drops are round, or roundish, and at the margin of the colony tend to lie along it in elongated shapes, which often, in old colonies, surround the greater part of the periphery of the colony. The largest drop found in the microscopical preparations is $30\,\mu$ in diameter.

The extracellular oil drops are also observed in one, two, three, and even four month old streak-cultures.

The intracellular oil-drops are at first minute in young cells but gradually grow larger according to the development of the cell and finally become very large (within two weeks). In the three day old colonies, in which the formation of extracellular oil is recognizable, every cell shows only small intracellular oil-drops.

The extracellular oil drops are yellowish and refractive like the intracellular ones.

The microchemical properties of the extracellular drops, as follows, prove their oily nature. The extracellular drops stain orange-red with Sudan III, red with alkannin or with scharlach R, and blue with cyanin, but are inert to osmium tetroxide. While these reactions to the dyestuffs are in common with the intracellular oil, the inertness to osmium tetroxide is different, as the latter blackens itself in reducing it. If the extracellular drops are treated with a mixture of a concentrated solution of potassium hydroxide and ammonia water (8), needle-shaped crystals of soap are formed. They are not soluble in cold or hot water but are soluble in all the following common solvents for oil, i. e., petroleum-ether, ether, acetone, chloroform, carbon disulphide, benzene, and xylene, and are, moreover, soluble in alcohol — like castor-oil.

The composition of the culture medium influences the formation of the extracellular oil.

When the yeasts are cultivated in a culture solution of the same composition as before but free from agar, i. e., Knop's solution with 10% saccharose, in which this yeast forms pink sediment and slight pink flecks on the surface of the turbid liquid, no remarkable difference in the formation of the extracellular oil, as well as in the development of the intracellular oil and in the morphology of the cells, is observed.

In the presence of various organic components (besides agar) in the culture medium instead of 10% saccharose, the development of the extra- and intracellular oils is as follows:

Organic component		Development of extracellular oil	Development of intracellular oil	Growth
Saccharose (the usual)	10%	++++	+++	++++
Saccharose	2%	+++	+++	++++
Dextrose	2%	++	+++	++++
Maltose	2%	+	++	++++
Lactose	2%	—	+	+
Starch (soluble)	2%	—	+	+
Dextrin	2%	—	+	+++
Glycerine	2%	—	+	+++
Mannite	2%	++	++	++++
Calcium acetate	2%	++	+++	+++
Tyrosine (nearly saturated)	0.04%	—	+	++
Asparagine	2%	—	+	++
Peptone	5%	—	+	+++
Saccharose 10% + peptone 1%		++	+++	++++

In the column "Development of extracellular oil," — shows no formation, and + formation, of which the increasing rate is indicated by the increasing numbers of +.

In that "Development of intracellular oil," +++ shows that the drops are large, ++ medium, and + small.

The method of plate-culture used here is quite the same as that usually employed in the present investigation, and the observation also lasted for two weeks.

The mode of the formation of the extracellular oil was judged by both direct observation of the colonies, intact and spread with a platinum wire, and in microscopical preparations.

"Growth" was compared with the growth on streak-cultures.

No remarkable change was noticed in the nature of the colonies and the cells in all the cases except in the case of 'Peptone 5%,' in which the cells are roundish and larger (8–12 μ long).

In the culture with calcium acetate, precipitation of crystals of calcium carbonate in the environment of the colonies took place, which phenomenon Molisch (9) closely investigated.*

As is shown in the table, the development of the extracellular oil is parallel to that of the intracellular oil and, moreover, to the rate of growth in general.

In the literature within the scope of my knowledge, only one species of yeast which forms extracellular oil is described, *Torula rubra* Schimon (10, 11). *Torula rubra*, which was found in the reserve water in a brewery, resembles my yeast but further close investigation is necessary to determine whether they are identical or not. Besides *Torula rubra*, Schimon described another, *Torula sanguinea* Schimon (10, 11), which was isolated from a beer. *Torula sanguinea* has many characters in common with *T. rubra* but is especially distinguished from the latter in its lacking a slime-halo and by the non-formation of extracellular oil, as well as by the weaker development of the intracellular oil. Therefore it resembles my yeast No. 20.

SUMMARY.

1. The author investigated the nectar of 273 flowers of twenty-three species of plants which were blooming in the city of Sendai, Japan, and its neighbourhood, during April to June 1925, and 120 (44%) flowers belonging to almost all of the species were found to be infected by yeasts which could be distinguished in twenty-two forms, ten forms of which were scarce.

2. The same forms of yeasts were found in the nectar of various plants and in different places, not only in the town and in the field but even on the high mountain. No definite relation between the species of plant and both the rate of yeast infection and the form of yeast could be recognized.

3. The rate of infection increased with the seasonal rise in temperature.

4. Among the twenty-two forms, two forms are colourless, two yellowish, ten white, one yellowish white, one orange, five pink, and one chocolate-coloured.

---

*) This form of yeast, named No. 19, had been first isolated by me from the flower nectar of *Antirrhinum majus* blooming in the garden of the Institute in September 1924, and the experiments concerning this yeast were carried out under the direction of Professor H. Molisch during his stay in Sendai.

5. Ascospore formation has never been observed.

6. Alcohol fermentation is not manifest with dextrose, saccharose, maltose, and lactose.

7. So-called "Kreuzform" was seldom found in the nectar, never in the culture. It was assumed by two forms of yeasts.

8. A pink form is peculiar in that it forms a considerable amount of oil out of the cell, as well as intracellular oil-drops. This is contrary to the common yeasts except *Torula rubra* SCHIMON, which resembles this form. It is a question for the time being, however, whether they are identical or not. The extracellular oil is formed in Knop's solution with agar in the presence of saccharose, dextrose, maltose, mannite, or calcium acetate, never in the presence of lactose, starch, dextrin, glycerine, tyrosine, asparagine, or peptone. The development of the extracellular oil is parallel to that of the intracellular oil and to the rate of growth.

BIBLIOGRAPHY.

1) REUKAUF, E. 1911–12. Nektarhefen. Die Kleinwelt. Jg. 3, pp. 25–27.
2) STOLTZ, 1911–12. Sprosspilze im Nektar der Blüten. Mikrokosmos. V, pp. 202–206.
3) HILKENBACH, R. 1911. Nektarhefen. Neue Beiträge zur Kenntnis der wilden Hefen in der Natur. Dissertation. Kiel.
4) SCHUSTER, V. u. ULEHLA, V. 1913. Studien über Nektarorganismen. (Vorläufige Mitteilung.) Ber. d. deutsch. bot. Gesellsch. Bd. 31, pp. 129–138.
5) SCHOELLHORN, K. 1919. Über die Gärung einiger Nektarhefen von Winterpflanzen. Dissertation. Genf.
6) HAUTMANN, F. 1924. Über die Nektarhefe *Anthomyces Reukaufii*. Arch. f. Protistenk. Bd. 48, pp. 213–244.
7) PRINGSHEIM, E. u. BILEWSKY, H. 1911. Über Rosahefe. Beitr. zur Biol. d. Pflanz. Bd. 10, p. 118.
8) MOLISCH, H. 1923. Mikrochemie der Pflanze. 3. Aufl. Jena. p. 119.
9) MOLISCH, H. 1925. Über Kalkbakterien und andere kalkfällende Pilze. Centralbl. f. Bakt. Abt. II, Bd. 65, p. 137.
10) SCHIMON, O. 1911. Beiträge zur Kenntnis rotgefärbter niederer Pilze. Dissertation. München.
11) SAITO, K. 1923. Untersuchungen über die atmospharischen Pilzkeime. III. Mitteilung. Japan. Journ. of Bot. Vol, 1. pp. 1–54.

EXPLANATION OF PLATES I & II.

The typical cells of every form. 1200/1.

The number of the figure corresponds to the number of the form.

The circles in full lines in the cell show the oil-drops, those in dotted lines the vacuoles. The dotted line around the outline of the cell indicates the outline of the slime-halo.

Fig. 13. a) Normal cells. b) Durable cells.

Fig. 16. a) Young cells from a three day old colony. b) Older cells from a seven day old colony. c) Old cells from a fourteen day old colony.

Fig. 17. a) Young cells from a three day old colony. b) Older cells from a five day old colony. c) Old cells from a fourteen day old colony.

Fig. 18. a) Younger cells from a seven day old colony. b) Old cells from a fourteen day old colony.

Fig. 19. a) Young cells from a three day old colony. b) Older cells from a five day old colony. c) Old cells from a fourteen day old colony.

Fig. 20. a) Younger cells from a five day old colony. b) Old cells from a fourteen day old colony.

In the other figures only old cells from fourteen day old colonies are shown.

TABLE I. Plants investigated.

1.	*Erythronium japonicum*, MAKINO.	(Liliaceae)
2.	*Heloniopsis orientalis*, TANAKA.	
3.	*Hemerocallis disticha*, DONN. var. *Kwanso*, NAKAI.	
4.	*Iris Pseudacorus*, L.	(Iridaceae)
5.	*Iris sanguinea*, DONN.	
6.	*Aquilegia flabellata*, SIEB. et ZUCC.	(Ranunculaceae)
7.	*Aquilegia* (hybrid)	
8.	*Epimedium macranthum*, MORR. et DECNE. var. *violaceum*, FR.	(Berberidaceae)
9.	*Chaenomeles lagenaria*, KOIDZ.	(Rosaceae)
10.	*Prunus Persica*, SIEB. et ZUCC. var. *vulgaris*, MAXIM.	
11.	*Camellia japonica*, L. var. *hortensis*, MAKINO.	(Theaceae)
12.	*Viola grypoceras*, A. GRAY.	(Violaceae)
13.	*Viola tricolor*, L.	
14.	*Viola violacea*, MAKINO.	
15.	*Shortia soldanelloides*, MAKINO, var. *genuina*, MAKINO, f. *typica*, MAKINO.	(Diapensiaceae)
16.	*Rhododendron Kaempferi*, PLANCH.	(Ericaceae)
17.	*Rhododendron nikoense*, NAKAI.	
18.	*Primula japonica*, A. GRAY.	(Primulaceae)
19.	*Antirrhinum majus*, L.	(Scrophulariaceae)
20.	*Digitalis purpurea*, L.	
21.	*Diervilla japonica*, DC.	(Caprifoliaceae)
22.	*Lonicera gracilipes*, MIQ. var. *glabra*, MIQ.	
23.	*Lonicera sempervirens*, AIT.	

TABLE II. Distribution of the yeasts.

*) In this column, a, b, c....indicate each individual flower of the same plant lodging yeasts.

**) In this column, + means (scarce), ++ (many), and +++ (numerous).

***) In the plants thus marked I investigated inflorescences, mixing the nectar from all the flowers of each inflorescence.

Plants examined	Locality	Date	Number of flowers examined	Number of flowers lodging yeasts	Form of yeast	Number of flower lodging each kind of yeast*	Frequency of occurrence of yeast**
Erythronium	C	Apr. 28	9	3	No. 7 No. 18	3 1	+ +
	D	May 8	2	1	No. 7	1	++
			7	2	No. 7 No. 13 No. 18	1a 1b 1a	++ ++ +
*Heloniopsis****	C	Apr. 20	2	0	.		
		Apr. 22	5	0			
		Apr. 28	3	3	No. 1 No. 2 No. 15 No. 20 No. 4	1a 1b 1b 1b 1c	+++ +++ +++ +++ +++
		Apr. 22	5	0			
		Apr. 28	3	2	No. 2 No. 18	2 1	+++ +++
Hemerocallis	C	June 17	5	5	No. 2 No. 13	1 5	+++ +++
			5	1	No. 16	1	+
Iris Pseud.	A	June 9	8	8	No. 13 No. 18 No. 19	8 1a 1b	+++ + +++
Iris sanguin.	B	June 11	8	7	No. 13 No. 15 No. 20	7 1a 1a	+++ ++ ++
Aquilegia flabel.	A	May 13	6	3	No. 3 No. 18 No. 19	1a 1b 1c	+ + +++

Plants examined	Locality	Date	Number of flowers examined	Number of flowers lodging yeasts	Form of yeast	Number of flower lodging each kind of yeast*	Frequency of occurrence of yeast**
Aquilegia (hybrid)	B	June 2	10	5	No. 2	1a 1b	+++ +++
					No. 17	1c	++
					No. 18	2d 1a	+++ +
		June 11	7	7	No. 13	7	+++
*Epimedium****	C	May 14	9	1	No. 7	1	++
	D	May 3	1	0			
			5	0			
Chaenomeles	A	Apr. 29	4	1	No. 4	1	+
		May 13	3	2	No. 3 No. 7 No. 15 No. 18 No. 19	1a 1a 1a 1a 1b	+++ +++ +++ +++ +
	B	Apr. 29	5	0			
		May 2	10	1	No. 18	1	+++
		May 14	2	2	No. 11 No. 13 No. 14 No. 18	1a 1a 1b 1b	+++ +++ ++ +
	D	May 3	7	1	No. 11	1	+++
Prunus	A	Apr. 29	5	1	No. 2	1	+
Camellia	B	May 2	5	0			
		May 14	5	0			
	C	May 14	4	4	No. 8 No. 12	3a 1b	+++ +++
Viola gryp.	A	Apr. 22	5	1	No. 6	1	+
	C	Apr. 28	5	2	No. 7 No. 18	1 1	+ +
	D	May 3	5	1	No. 7	1	++
			4	0			

Plants examined	Locality	Date	Number of flowers examined	Number of flowers lodging yeasts	Form of yeast	Number of flower lodging each kind of yeast*	Frequency of occurrence of yeast**
Viola tric.	A	June 2	10	8	No. 2	1a	+++
					No. 7	1b	+++
					No. 13	2d	+++
					No. 15	1c	++
					No. 19	1a 1b 1c 3e	+++ +++ ++ +++, ++
Viola viol.	C	Apr. 28	5	3	No. 2	1a	+++
					No. 10	1b	+
					No 18	1c	+++
*Shortia****	E	June 27	5	1	No. 17	1	+++
			3	1	No. 7	1	++
Rhododendron Kaempff.	C	May 14	8	2	No. 9	1a	++
					No. 11	1b	++
Rhododendron nik.	E	June 27	6	1	No. 12	1	+++
Primula	A	June 5	2	1	No. 11	1	+++
Antirrhinum	A	Apr. 24	9	1	No. 3	1	+
		Apr. 29	8	3	No. 2	1a	++
					No. 4	1b	+
					No. 7	1c	+
					No. 18	1a	+
		May 13	2	2	No. 5	1a	++
					No. 18	1b	+++
					No. 19	1a	++
		June 13	8	7	No. 13	1a	+++
					No. 19	5c 1a	+++ +++
					No. 20	1b	+++
Digitalis	A	June 13	9	8	No. 3	1a 1b 3f	++ ++ ++, +++
					No. 4	1d 1c	+ +
					No. 18	1c 3f	+ +, ++, +++
					No. 19	1b 1a	+ ++
					No. 21	1e	+++
					No. 22	1a	++

Plants examined	Locality	Date	Number of flowers examined	Number of flowers lodging yeasts	Form of yeast	Number of flower lodging each kind of yeast*	Frequency of occurrence of yeast**
Diervilla	A	June 5	7	7	No. 3	1a	++
					No. 13	4d 1a 1b	+++ +++ +++
					No. 19	1c	+++
					No. 20	1b	+
	E	June 27	4	3	No. 11	3	+++
					No. 20	1	++
Lonicera grac.	A	Apr. 29	5	1	No. 18	1	+
		May 13	7	7	No. 11	2a	+++
					No. 12	5b	+++
Lonicera semp.	A	June 9	6	0			

TABLE III.

Distribution of each form of yeast.

Form of yeast	Plant lodging the yeast	Location of plant	Date	Number of flowers lodging the yeast	Total number of plants	Total number of flowers	Locality of the yeast
No. 1	*Heloniopsis*	C	Apr. 28	1	1	1	C
No. 2	*Heloniopsis*	C	Apr. 28	3	7	10	A, B, C
	Hemerocallis	C	June 17	1			
	Aquilegia (hybrid)	B	June 2	2			
	Prunus	A	Apr. 29	1			
	Viola tric.	A	June 2	1			
	Viola viol.	C	Apr. 28	1			
	Antirrhinum	A	Apr. 29	1			
No. 3	*Aquilegia flabel.*	A	May 13	1	5	9	A
	Chaenomeles	A	May 13	1			
	Antirrhinum	A	Apr. 24	1			
	Digitalis	A	June 13	5			
	Diervilla	A	June 5	1			

Form of yeast	Plant lodging the yeast	Location of plant	Date	Number of flowers lodging the yeast	Total number of plants	Total number of flowers	Locality of the yeast
No. 4	*Heloniopsis*	C	Apr. 28	1	4	5	A, C
	Chaenomeles	A	Apr. 29	1			
	Antirrhinum	A	Apr. 24	1			
	Digitalis	A	June 13	2			
No. 5	*Antirrhinum*	A	May 13	1	1	1	A
No. 6	*Viola gryp.*	A	Apr. 22	1	1	1	A
No. 7	*Erythronium*	C	Apr. 28	3	7	12	A, C, D, E
		D	May 3	2			
	Epimedium	C	May 14	1			
	Chaenomeles	A	May 13	1			
	Viola gryp.	C	Apr. 28	1			
		D	May 3	1			
	Viola tric.	A	June 2	1			
	Shortia	E	June 27	1			
	Antirrhinum	A	Apr. 29	1			
No. 8	*Camellia.*	C	May 14	3	1	3	C
No. 9	*Rhododendron Kaempf.*	C	May 14	1	1	1	C
No. 10	*Viola viol.*	C	Apr. 28	1	1	1	C
No. 11	*Chaenomeles*	B	May 14	1	5	9	A, B, C, D, E
		D	May 3	1			
	Rhododendron Kaempf.	C	May 14	1			
	Primula	A	June 5	1			
	Diervilla	E	June 27	3			
	Lonicera grac.	A	May 13	2			
No. 12	*Camellia*	C	May 14	1	3	7	A, C, E
	Rhododendron nik.	E	June 27	1			

Form of yeast	Plant lodging the yeast	Location of plant	Date	Number of flowers lodging the yeast	Total number of plants	Total number of flowers	Locality of the yeast
No. 12	*Lonicera grac.*	A	May 13	5	3	7	A, C, E
No. 13	*Erythronium*	D	May 3	1	9	38	A, B, C, D
	Hemerocallis	C	June 17	5			
	Iris Pseud.	A	June 9	8			
	Iris sanguin.	B	June 11	7			
	Aquilegia (hybrid)	B	June 11	7			
	Chaenomeles	B	May 14	1			
	Viola tric.	A	June 2	2			
	Antirrhinum	A	June 13	1			
	Diervilla	A	June 5	6			
No. 14	*Chaenomeles*	B	May 14	1	1	1	B
No. 15	*Heloniopsis*	C	Apr. 28	1	4	4	A, B, C
	Iris sanguin.	B	June 11	1			
	Chaenomeles	A	May 13	1			
	Viola tric.	A	June 2	1			
No. 16	*Hemerocallis*	C	June 17	1	1	1	C
No. 17	*Aquilegia* (hybrid)	B	June 2	1	2	2	B, E
	Shortia	E	June 27	1			
No. 18	*Erythronium*	C	Apr. 28	1	11	20	A, B, C, D
		D	May 3	1			
	Heloniopsis	C	Apr. 28	1			
	Iris Pseud.	A	June 9	1			
	Aquilegia flabel.	A	May 13	1			
	Aquilegia (hybrid)	B	June 2	3			
	Chaenomeles	A	May 13	1			
		B	May 2	1			

Form of yeast	Plant lodging the yeast	Location of plant	Date	Number of flowers lodging the yeast	Total number of plants	Total number of flowers	Locality of the yeast
No. 18	*Chaenomeles*	B	May 14	1	11	20	A, B, C, D
	Viola gryp.	C	Apr. 28	1			
	Viola viol.	C	Apr. 28	1			
	Antirrhinum	A	Apr. 29	1			
			May 13	1			
	Digitalis	A	June 13	4			
	Lonicera grac.	A	Apr. 29	1			
No. 19	*Iris Pseud.*	A	June 9	1	7	19	A
	Aquilegia flabel.	A	May 13	1			
	Chaenomeles	A	May 13	1			
	Viola tric.	A	June 2	6			
	Antirrhinum	A	May 13	1			
			June 13	6			
	Digitalis	A	June 13	2			
	Diervilla	A	June 5	1			
No. 20	*Heloniopsis*	C	Apr. 28	1	4	5	A, B, C, E
	Iris sanguin.	B	June 11	1			
	Antirrhinum	A	June 13	1			
	Diervilla	A	June 5	1			
		E	June 27	1			
No. 21	*Digitalis*	A	June 13	1	1	1	A
No. 22	*Digitalis*	A	June 13	1	1	1	A

TABLE IV.

Rates of yeast infection with regard to the locality and the month.

Locality	Month	Number of plants examined	Number of plants lodging yeasts	Number of flowers examined	Number of flowers lodging yeasts		Plants examined
					Number	%	
A	April	5	5	36	8	22	*Chaenomeles, Prunus, Viola gryp, Antirrhinum, Lonicera grac.*
	May	4	4	18	14	78	*Aquilegia flabel., Chaenomeles, Antirrhinum, Lonicera grac.*
	June	7	6	50	39	78	*Iris Pseud., Viola tric., Primula, Antirrhinum, Digitalis, Diervilla, Lonicera semp.*
B	April	1	0	5	0	0	*Chaenomeles.*
	May	2	1	22	3	14	*Chaenomeles, Camellia.*
	June	2	2	25	19	76	*Iris sanguin., Aquilegia*(hyb).
C	April	4	4	37	13	35	*Erythronium, Heloniopsis, Viala gryp., Viola viol.*
	May	3	3	21	7	33	*Epimedium, Camellia, Rhododendron Kaempf.*
	June	1	1	10	6	60	*Hemerocallis.*
D	May	4	3	31	5	16	*Erythronium, Epimedium, Chaenomeles, Viola gryp.*
E	June	3	3	18	6	33	*Shortia, Rhododendron nik., Diervilla.*

TABLE V.

Rates of yeast infection with regard to the month.

Month	Number of flowers examined	Flowers lodging yeasts	
		Number	%
April	78	21	27
May	92	29	32
June	103	70	68

A. Table VI. Description of each form of yeast.

Form of yeast				No. 1	No. 2	No. 3
Plate-culture	Colony	Colour		Colourless	Colourless	Yellowish
		Lustre		Lustrous	Lustrous	Lustrous
		Transparency		Transparent	Transparent	Translucent
		Form		Circular	Circular	Circular
		Elevation		Convex	Convex	Convex
		Edge		Entire	Entire	Entire
		Consistency		Slimy	Slimy	Slimy
		Edge of deep-seated colony				
	Cell	Form		Round	Ellipsoidal, often oval	Roundish or round
		Size	Length	7–8 μ, seldom 6–10 μ	3–6 μ*, seldom 10 μ	6–7 μ
			Breadth		2–4 μ*, seldom 5 μ	
		Slime-halo (maximum thickness)		10 μ	8 μ	3 μ
		Oil-drop (max. size)		Small	Large	Small
		Specific character				
Streak-culture				Edge: entire; later, finely crenate	Edge: entire	Edge: crenate
Stab-culture				Short, irregular projections from stab	Short, irregular projections from stab	Short, irregular projections from stab
Growth				Medium	Strong	Medium

*) Measurement was made of singular cells instead of mother cells.

No. 4	No. 5	No. 6	No. 7	No. 8
Yellowish	White	White	White	White
Lustrous	Lustrous	Lustrous	Lustrous	Lustrous
Translucent	Opaque	Opaque	Opaque	Opaque
Circular	Circular	Circular	Circular	Circular
Convex	Convex	Convex	Convex	Convex
Entire	Entire	Entire	Entire	Entire
Slimy	Slimy	Slimy	Slimy	Slimy
Ellipsoidal, often roundish	Roundish or round	Roundish, often ellip., oval or narrow at the middle	Ellipsoidal or roundish	Ellipsoidal
4–6 µ, seldom 7 µ	4–6 µ	6–10 µ (in the roundish), –14 µ (in the others)	3–6 µ*	3–4 µ, seldom 6 µ
3–5 µ, seldom 6 µ			2–4 µ*	2–3 µ
2 µ	Lacking	2 µ	4 µ	Lacking
Medium	Very large	Small	Small	Small
Edge: crenate	Edge: crenate	Edge: crenate	Edge: crenate	Edge: crenate
Short, irregular projections from stab	Short, irregular projections from stab	Short, irregular projections from stab	Short, irregular projections from stab	Short, irregular projections from stab
Medium	Weak	Weak	Weak	Weak

*) Measurement was made of singular cells instead of mother cells.

B.

Form of yeast				No. 9	No. 10	No. 11
Plate-culture	Colony	Colour		White	White	White
		Lustre		Lustrous	Lustrous	Lustrous
		Transparency		Opaque	Opaque	Opaque
		Form		Circular	Circular	Circular
		Elevation		Convex	Convex	Convex
		Edge		Entire	Entire	Entire
		Consistency		Slimy	Slimy	Slimy
		Edge of deep-seated colony				With branch-like projections
	Cell	Form		Ellipsoidal or oval	Elongated ellip. or elongated oval	Ellipsoidal, seldom oval or roundish
		Size	Length	5–7 μ, seldom 4–8 μ	6–12 μ	5–6 μ
			Breadth	3–4 μ	2–3 μ	3-5 μ
		Slime-halo (maximum thickness)		Lacking	Lacking	Lacking
		Oil-drop (max. size)		Small	Small	Small
		Specific character				
Streak-culture				Edge: crenate	Edge: crenate	Edge: crenate
Stab-culture				Short, irregular projections from stab	Short, irregular projections from stab	Short, irregular projections from stab
Growth				Weak	Weak	Weak

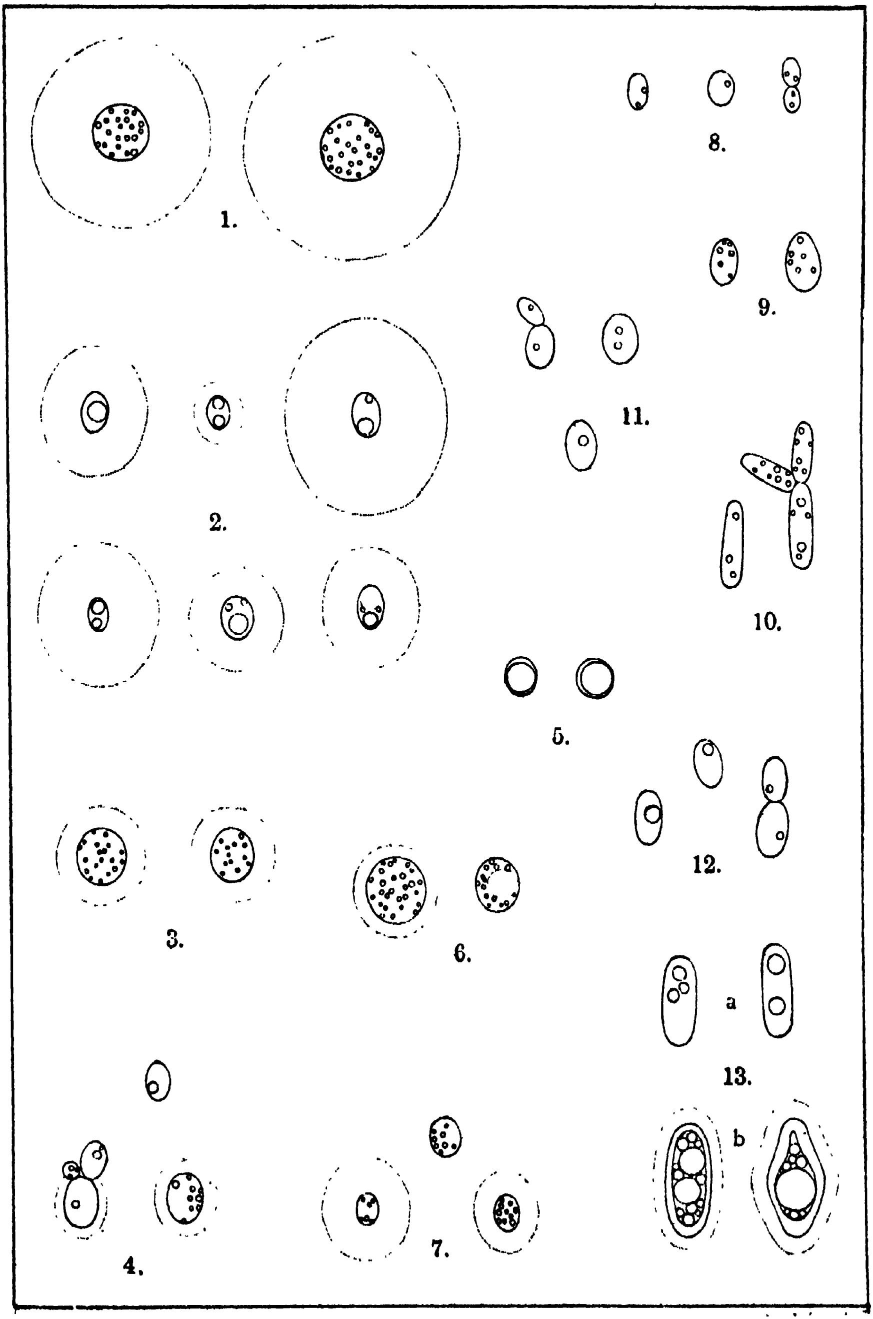
No. 12	No. 13	No. 14	No. 15	No. 16
White	White	Yellowish white	White	Orange
Lustrous	Lustrous	Lustrous	Weakly lustrous	Weakly lustrous
Opaque	Opaque	Opaque	Opaque	Opaque
Circular	Radiates branch-like projections (also in agar)	Circular	Circular	Circular
Convex	Convex	Convex	Drop-like	Convex
Entire		Entire	Entire	Entire
Slimy	Slimy	Slimy	Slimy	Slimy
With branch-like projections	With branch-like projections			
Ellipsoidal or elongated ellip., seldom oval or roundish	Elongated ellip.	Ellipsoidal or roundish	Ellip., oval, elongated ellip. or elong. oval, often tubular, seldom roundish, narrow at the middle or irregular	Roundish, often round or ellip., seldom oval or narrow at the middle
5–8 μ, seldom 9 μ	8–12 μ	5–8 μ	6–16 μ, seldom 30 μ	4–7 μ, seldom 9 μ
2–5 μ, seldom 8 μ	3–4 μ	4–6 μ	3–6 μ, seldom 7 μ	
Lacking	Lacking	2 μ	Lacking	Lacking
Medium	Large	Very large	Large	Very large
	Later forms many durable-cells*			
Edge: crenate	Radiates branch-like projections (also in agar)	Edge: entire	Edge: entire	Edge: entire Surface: later radially striped
Short, irregular projections from stab	Branch-like projections from stab	Short, irregular projections from stab	Short, irregular projections from stab	Short, irregular projections from stab
Weak	Weak	Strong	Strong	Strong

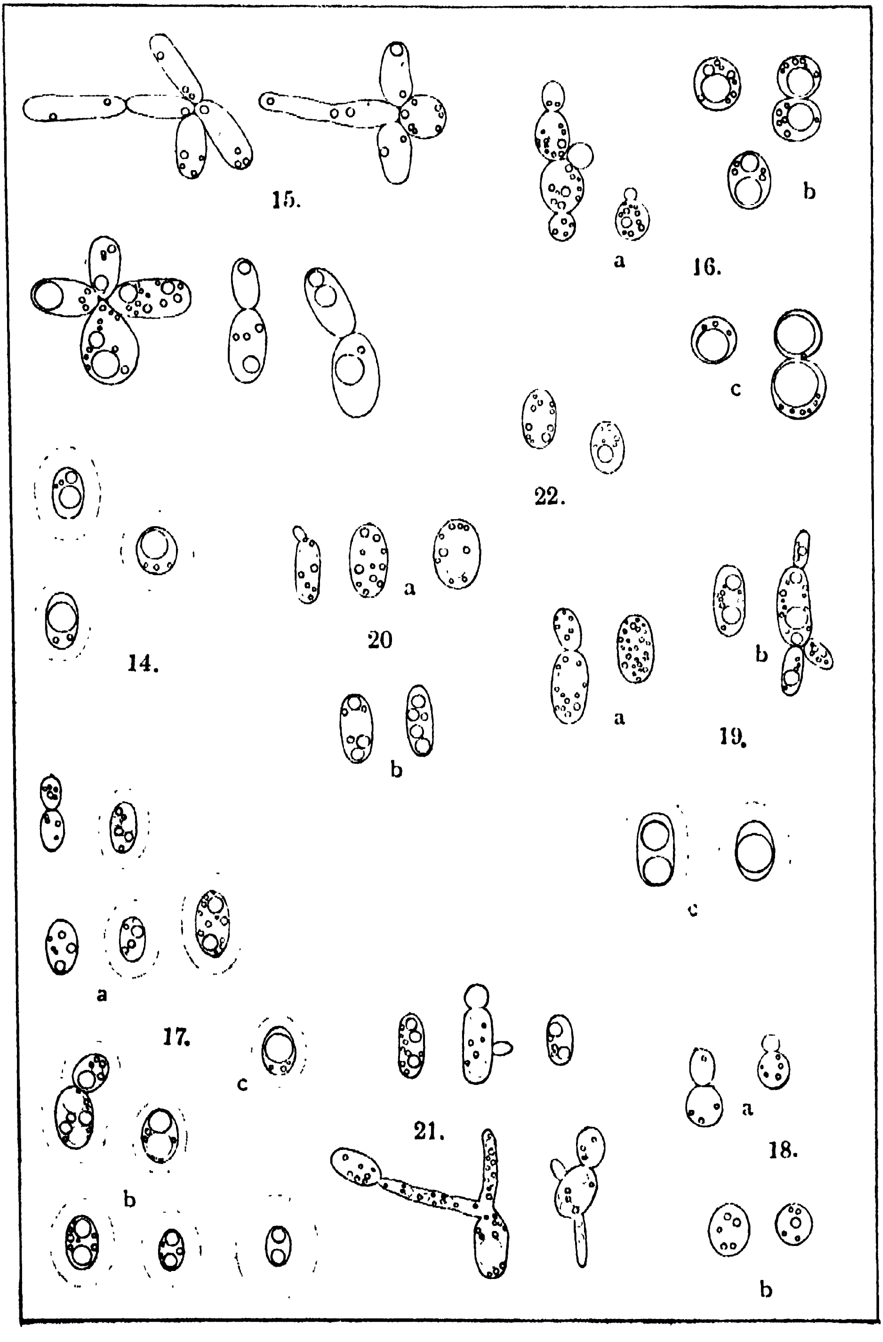
*) Form: ellip., elong. oval, or tapered off at both ends; 12–18 μ long, 5–7 μ broad; with thick cell wall; mostly with slime-halo (3 μ thick in maximum); filled with many small and large oil-drops.

C.

Form of yeast				No. 17	No. 18	No. 19
Plate-culture	Colony	Colour		Light pink	Pink	Pink
		Lustre		Lustrous	Lustrous	Lustrous
		Transparency		Transparent	Opaque	Opaque
		Form		Circular	Circular	Circular
		Elevation		Convex	Convex	Convex
		Edge		Entire	Entire	Entire
		Consistency		Slimy	Slimy	Slimy
		Edge of deep-seated colony				
	Cell	Form		Ellipsoidal, seldom oval or roundish	Ellipsoidal or roundish	Ellipsoidal, often elong. ellip., seldom oval, roundish, narrow at the middle or tubular
		Size	Length	4–8 μ	4–6 μ	6–9 μ, seldom 10 μ, –16 μ (in the tubular)
			Breadth	2–5 μ	3–5 μ	3–5 μ
		Slime-halo (maximum thickness)		4 μ	Lacking	2 μ
		Oil-drop (max. size)		Very large	Small	Very large
		Specific character				Forms extra-cellular oil
Streak-culture				Edge: entire	Edge: entire	Edge: entire
Stab-culture				Short, irregular projections from stab	Short, irregular projections from stab	Short, irregular projections from stab
Growth				Medium	Weak	Strong

No. 20	No. 21	No. 22
Pink	Pink	Lightly chocolate-coloured
Lustrous	Mat	Weakly lustrous
Opaque	Opaque	Opaque
Circular	Nearly circular	Circular
Convex	With concentric & radial ridges	Convex
Entire	Crenate	Entire
Slimy	Butter-like	Slimy
Ellipsoidal, often elong. ellip., seldom oval, roundish, narrow at the middle or tubular	Ellipsoidal, often thin & tubular (among bud-cells) or irregular ellip., seldom roundish, oval, elong. ellip. or narrow at the middle	Ellipsoidal, seldom narrow at the middle
5–10 μ, –16 μ (in the tubular)	5–9 μ, –25 μ (in the tubular bud-cells)	6–7 μ, seldom 8 μ
3-6 μ	3-6 μ	4 μ
Lacking	Lacking	Lacking
Medium	Medium	Medium
	Buds in any direction	
Edge: entire	Edge: entire Surface: wrinkled	Edge: entire Agar is chocolate-coloured near the colony.
Short, irregular projections from stab	Short, irregular projections from stab	Short, irregular projections from stab. Agar is likewise coloured.
Strong	Strong	Strong


1.
8.
9.
11.
2.
10.
5.
12.
3.
6.
a
13.
b
4.
7.


15.
a
b
16.
c
22.
14.
a
20
b
a
b
19.
c
a
17.
c
21.
b
a
18.
b

Triploidy of Chromosomes in Garden Varieties of *Primula Sieboldii*, E. MORR.

BY

MASAJIRO IINUMA.

In Japan there are about 20 species of wild primroses, among which *Primula Sieboldii* is most commonly cultivated in Tokyo and has many beautiful garden varieties. These varieties differ from one another in many respects, for example, in the colour of the flowers, in the form of the leaves, and especially in general size. Fig. 1 will be enough to show how distinct are the differences in the size of the leaves of these different varieties.

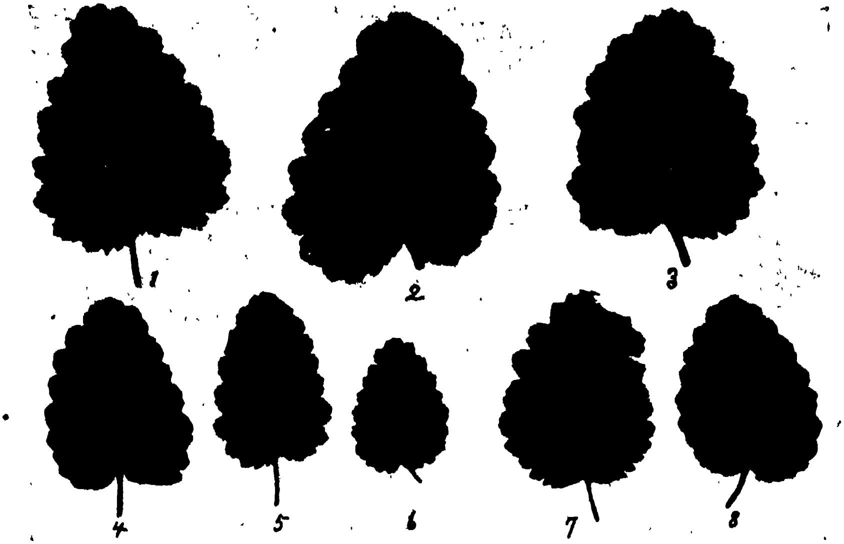


Fig. 1. Leaves of different varieties of *Primula Sieboldii*. 1. Shirowashi. 2. Nuresagi. 3. Sasanonami. 4. Mitanohikari. 5. Awobanofue. 6. Original form. 7. Suibijin. 8. Kurozomegawa.

The so-called "gigas" forms have been already reported in another species of *Primula*, e. g. *P. sinensis* (GREGORY, 1914). According to the investigation of GREGORY, the gigantism of this plant is sometimes accompanied by tetraploidy of chromosomes in each of the somatic cells. The present investigation was primarily undertaken in supposition that similar cases would likely be found in certain giant varieties of our primrose. The fixation of materials was made in Flemming's Bonn-solution; in the case of flower buds the material was first immersed in Carnoy's fluid for 15 minutes.

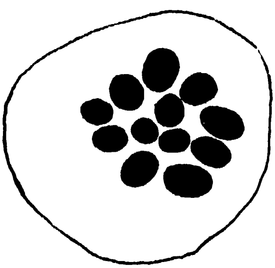


Fig. 2. Polar view of heterotype nuclear division in P. M. C. of wild *Primula Sieboldii*. (×4000)

In the first place, I examined the number of haploid chromosomes in the pollen mother-cells of wild specimens of *Primula Sieboldii*. This plant grows abundantly in the vicinity of Sendai. Fig. 2 shows the polar view of the heterotype nuclear division in these cells. From this figure one can conclude without any hestitation that the number of haploid chromosomes of this plant is 12.

The number of chromosomes in different species of *Primula* has already been reported by several authors. Their results can be summarized as follows:

Plant	Number of haploid chromosomes	Author
P. floribunda	9	DIGBY 1912, DAHLGREN 1916
P. verticillata	9	DIGBY 1912
P. kewensis	18	DIGBY 1912
P. officinalis	11	DAHLGREN 1916
P. sinensis	12	GREGORY 1909
P. sinensis var. *gigas*	24	GREGORY 1914

Additions to this list, according to a short study I made, are:

Plant	Haploid number	Diploid number
P. modesta var. *Fauriene*	9	—
P. japonica	22	—
P. nipponica	-	22
P. Reinii	—	24

In the above mentioned table it is noteworthy that the number of chromosomes of *P. japonica* is exactly the double of that of *P. officinalis*.

After this preliminary investigation, I entered upon the study of the number of chromosomes of garden varieties of *Primula Sieboldii*, of which 14 varieties were selected. Their Japanese names and numbers of chromosomes are as follows. The counts were made in root tips.

Name of varieties	Number of diploid chromosomes
Hiryu (飛龍)	24
Awohanofue (青葉ノ笛)	24
Mitanohikari (三田ノ光)	24
Suibijin (醉美人)	24
Edasango (枝珊瑚)	24
Kôkiden (光輝殿)	24
Hahanoai (母ノ愛)	24
Kurozomegawa (黒染川)	24
Uchiu (宇宙)	24
Hatsuhinode (初日ノ出)	24
Makinowo (槇ノ尾)	24
Shirowashi (白鷲)	36
Nuresagi (濡鷺)	36
Sasanonami (笹ノ浪)	36

The table shows that certain varieties have 36 somatic chromosomes, while the majority have 24. The number 36 corresponds exactly to the triploid chromosome number of the plant, whose diploid chromosome number is 24.

Triploidy of chromosomes in domesticated varieties has been reported recently in a number of plants and animals, namely in *Oenothera* (Stomps, 1912 a, b), *Morus* (Osawa, 1920), *Datura* (Blakeslee, Belling and Farnham, 1920), *Drosophila* (Bridges, 1921), *Hyacinthus* (de Mohl, 1921), *Canna* (Belling, 1922; Tokugawa and Kuwada, 1924), *Crepis* (Navashin, 1925), *Solanum* (Lesley and Mann, 1925), *Zea Mays* (Randolph and McClintoch, 1926).

As Fig. 3 shows, there is no perceptible difference between the size of the chromosomes of the "24" varieties and those of the "36" varieties, so the consideration that the "36" varieties are triploid mutants of *Primula Sieboldii* is justifiable.

As far as my investigation goes, there is no evidence for the existence of tetraploid mutants of this species, so it is reasonable to conclude that the triploidy in the present species has been produced by the union of the diploid germ cells with the normal haploid cells. The occurence of the diploid germ cells might have been caused by abnormal enviromental conditions, for example by a lowering or a rising of temperature during the progress of maturation division.

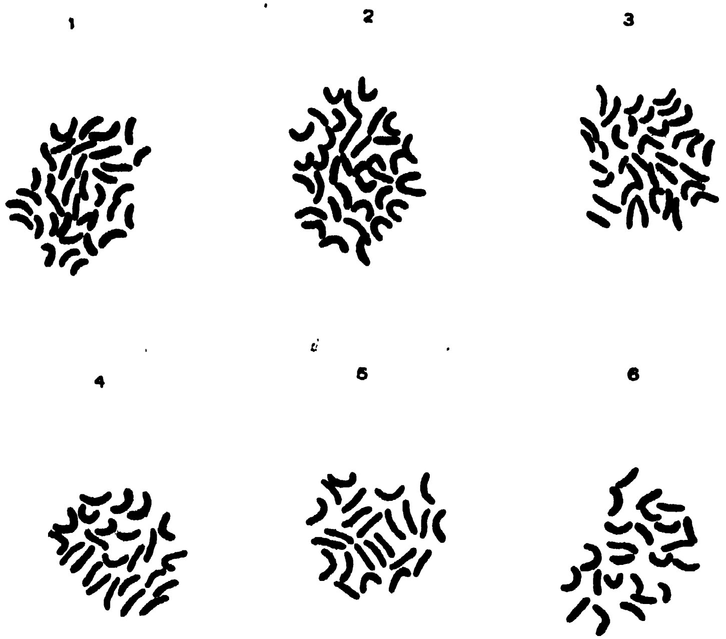


Fig. 3. Chromosomes in the root cells of certain varieties of *Primula Sieboldii*. (× 2000)
1. Shirowashi (triploid) 2. Nuresagi (triploid) 3. Sasaonami (triploid)
4. Hahanoai (diploid) 5. Edasango (diploid) 6. Kurozomegawa (diploid)

TOKUGAWA and KUWADA in their studies on *Canna* have made a comparison between the sizes of the cells of the triploid and the diploid varieties in the stomata of the leaves. In a similar way I also made measurements of the stomata of the triploid and the diploid varieties of *Primula Sieboldii*. The results are shown in the following table:

Length and Width of Stomata in μ

Name of plant	Length	Width	Average	
			Length	Width
Shirowashi (triploid)	43.5	35.0	50.5	37.0
	50.0	37.5		
	52.5	37.5		
	47.5	40.0		
	42.5	35.0		

Name of plant	Length	Width	Average	
			Length	Width
Shirowashi (triploid)	55.0	35.0	50.5	37.0
	55.0	40.0		
	55.0	37.5		
	55.0	37.5		
	50.0	35.0		
Sasanonami (triploid)	62.5	40.0	53.25	37.75
	52.5	35.0		
	52.5	40.0		
	55.0	35.0		
	52.5	37.5		
	47.5	37.5		
	50.0	32.5		
	60.0	45.0		
	50.0	37.5		
	50.0	37.5		
Nuresagi (triploid)	55.0	32.5	49.5	35.0
	55.0	37.5		
	45.0	37.5		
	50.0	35.0		
	50.0	35.0		
	45.0	35.0		
	50.0	37.5		
	47.5	32.5		
	50.0	32.5		
	47.5	35.0		
Mitanohikari (diploid)	50.0	37.5	47.0	33.75
	52.5	37.5		
	47.5	32.5		
	50.0	30.0		
	45.0	35.0		
	45.0	35.0		
	37.5	35.0		
	42.5	32.5		
	50.0	30.0		
	50.0	32.5		
Edasango (diploid)	45.0	32.5	44.25	34.00
	47.5	32.5		
	42.5	27.5		
	40.0	37.5		
	45.0	37.5		
	45.0	35.0		
	42.5	35.0		
	45.0	35.0		
	45.0	37.5		
	45.0	30.0		
Awobanofue (diploid)	42.5	35.0	42.00	33.00
	42.5	32.5		
	42.5	32.5		
	45.0	32.5		

Name of plant	Length	Width	Average	
			Length	Width
Awobanofue (diploid)	40.0	37.5	42.00	33.00
	45.0	35.0		
	42.5	32.5		
	37.5	30.0		
	37.5	32.5		
	45.0	30.0		
Hahanoai (diploid)	35.0	32.5	37.50	32.50
	37.5	30.0		
	40.0	32.5		
	37.5	35.0		
	32.5	30.0		
	35.0	30.0		
	35.0	30.0		
	40.0	35.0		
	37.5	32.5		
	45.0	37.5		
Kurozomegawa (diploid)	52.5	37.5	44.75	34.00
	40.0	35.0		
	45.0	35.0		
	42.5	35.0		
	42.5	35.0		
	45.0	32.5		
	45.0	35.0		
	45.0	27.5		
	45.0	32.5		
	45.0	35.0		
Uchiu (diploid)	50.0	37.5	44.75	34.25
	50.0	35.0		
	45.0	30.0		
	45.0	32.5		
	42.5	35.0		
	47.5	35.0		
	42.5	35.0		
	42.5	32.5		
	42.5	37.5		
	40.0	32.5		
Makinowo (diploid)	42.5	30.0	41.25	30.75
	40.0	30.0		
	42.5	32.5		
	37.5	32.5		
	47.5	32.5		
	42.5	30.0		
	42.5	30.0		
	35.0	30.0		
	40.0	27.5		
	42.5	32.5		

As the table shows, the stomata of the triploid varieties are much bigger than those of the diploid varieties. The general increase of the size of the cell in the triploid varieties is reflected in the enlargement of the whole organ. The leaves of the diploid and the triploid varieties have already been shown in Fig. 1. The meiotic division of the triploid varieties will be reported in a subsequent paper.

In conclusion, I wish to express my sincere thanks to Prof. M. TAHARA for his valuable advice and criticism throughout this work.

LITERATURE CITED.

1) BELLING, J. 1921. The Behavior of homologous chromosomes in a triploid *Canna*. Proc. Nat. Acad. of Science, Vol. 7, pp. 197–201.

2) BLAKESLEE, A. F., BELLING, J. and FARNHAM, M. E. 1920. Chromosomal duplication and Mendelian phenomena in *Datura* mutants. Science, N. S., Vol. 52., No. 1347, pp. 388–390.

3) BRIDGES, C. B. 1921. Triploid intersex in *Drosophila melanogaster*. Science, Vol. 54, pp. 252–254.

4) GREGORY, R. P. 1914. On the genetics of tetraploid plants in *Primula sinensis*. Pro. Royal Society, B. Vol. 87, pp. 484–492.

5) LESLEY, J. W., and MANN, M. C. 1925. Triploidy in the *Tomato*. Science. Vol. 61, No. 1573. pp. 208.

6) DE MOL, W. E. 1921. Over het voorkomen van heteroploide varieteiten van *Hyacinthus orientalis* L. in de hollandsche kulturen. Genetica. Vol. 3, pp. 97–192.

7) NAVASHIN, M. 1925. Polyploid mutation in *Crepis*. Triploid and pentaploid mutants of *Crepis capillaris*. Genetics. Vol. 10, No. 6, pp. 583–592.

8) OSAWA, I. 1920. Cytological and experimental studies in *Morus* with special reference to triploid mutants. Bull. Imp. Seric. Exp. Sta. Japan, Vol. 1, No. 3, pp. 318–369.

9) RANDOLPH, L. F., and McCLINTOCK, B. 1926. Polyploidy in *Zea Mays* L. The American Naturalist. Vol. 60, No. 666, pp. 99–102.

10) STOMPS, J. 1912. a) Die Entstehung von *Oenothera* gigas DE VRIES. Ber. d. Deutsch. Bot. Ges., Bd. 30, S. 406–416. 1912. b) Mutation bei *Oenothera biennis* L. Biologisches Centralblatt. Bd. 32. s. 521–535.

11) TOKUGAWA, Y., and KUWADA, Y. 1924. Cytological studies on some garden varieties of *Canna*. Japanese Jour. of Bot. Vol. 2, No. 3, pp. 157–173.

On the Growth Relation in Earshells.

By

KIICHIRO SASAKI.

(With 1 Plate)

In 1919, while I was staying at Miyako, Iwate-Ken, I had an opportunity of studying the growth relation in mature specimens of an earshell from four different localities of the same prefecture, Kosode, Taro, Omoi, and Ryori, and in 1924, while I was staying at Asamushi, Aomori-Ken, I had another opportunity to measure many mature and immature specimens of the same species.

According to KISHINOUYE (1895) the three following varieties of *Haliotis gigantea* are found in Japan and they are commonly called " Awabi " :

Haliotis gigantea CHEMNITZ	" Madaka "
Haliotis gigantea var. *mekai* KISHINOUYE	" Megai "
Haliotis gigantea var. *discus* REEVE	" Kuro " or " Yezoawabi "

The specimens, which I collected from the above mentioned places, were invariably identified as *Haliotis gigantea* var. *discus* by Prof. KISHINOUYE, to whom I wish to express my heartfelt thanks.

TABLE 1.

	Omoi	Taro	Kosode	Ryori	Asamushi
Shorter diam. / Longer diam.	71.290	70.810	70.120	69.490	67.510
Local ratio	1.000	0.993	0.984	0.975	0.947

Table 1 was made in order to compare the values of local ratios between the shorter and the longer diameters of the variety *discus* (Fig. 2). In the table, the results only from specimens of a size from 80 to 100 mm. in the longer diameter (that is the size of specimens sexually matured) are shown, and in determining the values of the local ratios, for convenience, the ratio for Omoi was taken as the unit. According to this table, the local ratio of the variety *discus*

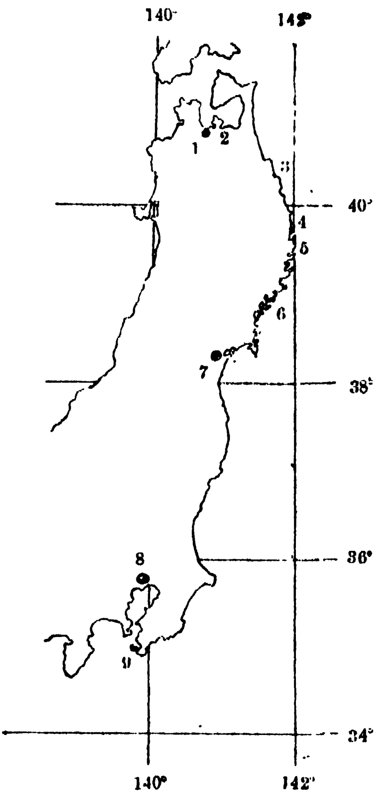


Fig. 1. Coast line of North Japan. 1, Aomori; 2, Asamushi; 3, Kosode; 4, Taro; 5, Omoi; 6, Ryori; 7, Sendai; 8, Tokyo; 9, Takanoshima.

shows a different value with the difference of localities, although this variety is described as a definite one.

Just at this time, E. NOMURA (1926c) of the same laboratory has published the results of his study on the growth relations in several molluscan shells. He states that "The growth relation between the height and the width of *Littorina, Limnaea, Viviparus,* and *Sphaerium* can be expressed by the equation $a=kb^x$, in which a and b are the measurements, k the local constant, and x the specific exponent," and that "the species, even from different localities, gives the same value for x all through, but it may give different values for k according to different localities." Thus it was the purpose of the present investigation to test the applicability of the exponential equation in expressing the growth relation in earshells, and to know, if possible, the relation between shell development and water temperature.

Before taking up the subject of this paper, I wish to express my sincere thanks to Prof. E. NOMURA for his kind supervision and for his helpful advice given during the progress of this investigation.

THE GROWTH RELATION BETWEEN THE SHORTER AND THE LONGER DIAMETER IN *Haliotis gigantea* VAR. *discus*.

In applying the equation $S=kL^x$, in which S denotes the shorter

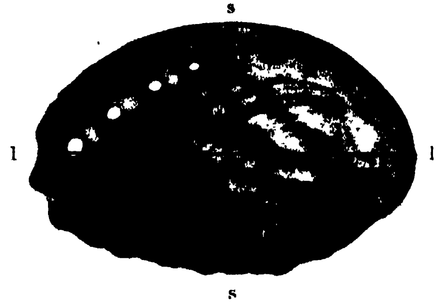


Fig. 2. *Haliotis gigantea* var. *discus* REEVE to illustrate the points at which measurements were taken. s–s, shorter diameter; l–l, longer diameter.

and L the longer diameter, for specimens from Omoi, Taro, Kosode, and Ryori (Tables 2–5 and Plate 1), the following results were obtained in the same way that NOMURA (1926b) adopted in the case of *Sphaerium heterodon* PILS.

$S = 1.406L^{0.85}$ for Omoi specimens
$S = 1.526L^{0.83}$ for Taro specimens
$S = 1.387L^{0.85}$ for Kosode specimens
$S = 1.302L^{0.86}$ for Ryori specimens

TABLE 2. Omoi

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
1	80.30	60.60	58.48	2.12
6	84.80	61.60	61.26	0.34
2	86.40	60.60	62.23	1.63
3	87.90	60.60	63.16	2.56
1	89.40	65.10	64.07	1.03
12	90.90	64.91	64.99	0.08
15	93.90	67.69	66.80	0.89
7	95.40	67.33	67.70	0.37
17	97.00	68.11	68.67	0.56
3	98.50	68.70	69.57	0.77
25	100.00	70.30	70.47	0.17
1	101.50	74.20	71.37	2.83
15	103.00	72.31	72.25	0.06
3	104.50	71.20	73.15	1.95
4	106.10	72.48	74.11	1.63
1	107.60	75.80	75.00	0.80
2	109.10	75.80	75.88	0.08
1	110.60	78.80	76.77	2.03
119				

TABLE 3. Taro

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
1	75.80	56.10	55.12	0.98
4	81.80	59.48	58.81	0.67
10	84.80	60.75	60.65	0.10
4	86.40	62.10	61.61	0.49
15	87.90	63.12	62.53	0.59
3	89.40	61.60	63.43	1.83
32	90.90	64.15	64.34	0.19
6	92.40	66.67	65.23	1.44
20	93.90	65.99	66.13	0.14
6	95.40	66.93	67.02	0.09
23	97.00	67.60	67.99	0.39
3	98.50	68.70	68.88	0.18
21	100.00	70.00	69.77	0.23
5	101.50	68.50	70.66	2.16
18	103.00	71.88	71.53	0.15
1	104.50	68.20	72.43	4.23
8	106.10	73.48	73.37	0.11
4	109.10	76.90	75.13	1.77
5	112.10	77.58	76.88	0.70
5	115.10	78.20	78.62	0.42
2	118.20	83.30	80.42	2.88
196				

TABLE 4. Kosode

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
1	78.80	57.60	56.77	0.83
1	80.30	57.60	57.69	0.09
1	81.80	59.10	58.60	0.50
1	83.30	60.60	59.52	1.08
4	84.80	61.73	60.43	1.30
2	86.40	61.35	61.39	0.04
1	87.90	63.60	62.30	1.30
1	89.40	60.60	63.21	2.61
1	90.90	62.10	64.11	2.01
3	92.40	64.10	64.99	0.89
1	93.90	62.10	65.90	3.80
2	95.40	68.95	66.78	2.17
3	97.00	63.60	67.74	4.14
4	98.50	68.95	68.63	0.32
5	100.00	69.40	69.52	0.08
3	101.50	70.70	70.40	0.30
1	103.00	75.80	71.28	4.52
2	104.50	72.70	72.17	0.53
1	106.10	72.70	73.11	0.41
1	110.60	75.80	75.73	0.07
1	113.60	78.80	77.48	1.32
40				

TABLE 5. Ryori

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
1	75.80	54.50	53.97	0.53
3	78.80	55.53	55.79	0.26
1	80.30	56.10	56.69	0.59
9	81.80	57.58	57.59	0.01
2	83.30	58.35	58.49	0.14
2	84.80	59.10	59.39	0.29
3	86.40	61.10	60.33	0.77
6	87.90	61.85	61.23	0.62
1	89.40	63.60	62.11	1.49
6	90.90	62.60	63.00	0.40
2	92.40	63.60	63.87	0.27
13	93.90	64.67	64.76	0.09
4	95.40	64.35	65.63	1.28
9	97.00	67.69	66.57	1.12
1	98.50	66.70	67.44	0.74
16	100.00	69.04	68.31	0.73
1	101.50	68.20	69.19	0.99
2	103.00	68.95	70.04	1.09
4	104.50	70.80	70.92	0.08
5	106.10	72.72	71.84	0.88
2	109.10	72.70	73.56	0.86
1	112.10	75.80	75.28	0.52
94				

Table 6 was made for determining the ratio between the local constants, the value of k from the Omoi specimens being taken, for convenience, as the unit. It may be seen in this table that the ratio between k at two localities is constant, even though the absolute values of k are different following the difference of the value of x.

TABLE 6.

	$x=0.83$		$x=0.85$		$x=0.86$	
	Value of k	Ratio between k	Value of k	Ratio between k	Value of k	Ratio between k
Omoi	1.541	1.000	1.406	1.000	1.343	1.000
Taro	1.526	0.990	1.392	0.990	1.330	0.990
Kosode	1.518	0.985	1.387	0.986	1.325	0.986
Ryori	1.492	0.968	1.363	0.969	1.302	0.969

EQUATIONS FOR SPECIMENS FROM ASAMUSHI.

From 1098 specimens from Asamushi the equation

$$S=0.810L^{0.86}$$

was obtained. This expression is entirely different from those which were determined for specimens from the four localities of Iwate-Ken. In this case, however, it must be noticed that both mature and immature specimens were measured collectively, while in the case of those from Iwate-Ken immature ones were omitted.

According to Kishinouye (1896) *Haliotis gigantea* var. *discus*

TABLE 7. Asamushi

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
1	35.30	26.20	24.71	1.49
6	37.17	25.95	25.98	0.03
4	37.83	26.15	26.42	0.27
7	39.16	27.37	27.33	0.04
16	39.98	27.83	27.88	0.05
18	40.97	28.46	28.55	0.09
20	42.06	29.42	29.29	0.13
42	43.06	30.17	29.96	0.21
38	43.98	30.42	30.58	0.16
43	44.87	31.09	31.18	0.09
42	45.95	31.88	31.92	0.04
49	46.97	32.34	32.60	0.26
37	47.99	32.94	33.29	0.35
57	49.01	33.77	33.97	0.20
45	50.01	34.66	34.64	0.02
39	50.99	35.45	35.30	0.15
52	51.98	35.84	35.97	0.13
45	53.00	36.72	36.65	0.07
30	53.97	37.27	37.30	0.03
39	54.96	37.95	37.97	0.02
40	56.00	38.55	38.66	0.11
41	57.00	39.22	39.33	0.11
37	57.88	39.71	39.92	0.21
24	58.97	40.85	40.65	0.20
31	59.84	41.21	41.23	0.02
20	60.97	41.78	41.99	0.21
32	61.93	42.82	42.63	0.19
28	62.92	43.32	43.29	0.03
25	64.07	43.87	44.05	0.18
18	64.91	44.63	44.61	0.02
18	65.86	45.33	45.24	0.09
16	66.88	46.00	45.93	0.07
14	67.91	46.19	46.62	0.48
9	69.01	46.84	47.35	0.51
13	70.09	47.29	48.06	0.77
14	70.91	48.21	48.61	0.40
6	71.88	49.88	49.26	0.62
9	72.86	50.08	49.91	0.17
7	73.87	50.53	50.58	0.05
7	74.94	51.37	51.29	0.08
1045				

TABLE 8. Asamushi

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
7	76.03	51.59	52.30	0.71
6	77.00	53.03	52.86	0.17
5	77.86	52.94	53.35	0.41
4	79.03	53.95	54.04	0.09
3	79.90	53.73	54.55	0.82
5	81.18	54.96	55.29	0.33
3	82.00	55.40	55.76	0.36
4	82.95	57.43	56.31	1.12
4	84.08	56.88	56.96	0.08
2	85.30	57.40	57.66	0.26
2	86.25	59.20	58.21	0.99
2	86.95	59.10	58.61	0.49
1	87.60	59.80	58.99	0.81
1	88.80	61.20	59.67	1.53
1	89.80	60.90	60.24	0.66
1	91.80	60.70	61.37	0.67
1	96.00	63.50	63.76	0.26
1	99.00	64.10	65.44	1.34
53				

sexually matures at Asamushi at the size of from 75.7 to 90.9 mm. in the longer diameter. Thus in assuming that the animal reached maturation at the size of 76 mm. and in grouping the specimens into the ones shorter (Table 7) and the ones longer (Table 8) than 76 mm., the following equations were obtained.

$S=0.779L^{0.97}$ for specimens shorter than 76 mm.

$S=1.317L^{0.85}$ for specimens longer than 76 mm.

According to these equations, the mature specimens, even from Asamushi, give the value 0.85 for x in near approximation to those given by specimens from Iwate-Ken. Thus, in summarizing the values of x from the five localities, we may infer that the value of the specific exponent for mature specimens of *Haliotis gigantea* var. *discus* may probably be 0.85, because we could obtain that value for 3 out of 5 cases.

As to the growth relations in *Sphaerium heterodon* PILS., *Littorina sitchana* PHIL., *Limnaea japonica* JAY, and *Viviparus japonicus* var. *iwakawai* PILS., NOMURA (1926c) says that "the molluscans show a growth relation between the height and the width which may be expressed by $a=kb$ while they are young or during their life," and again the equation $S=kL^{0.97}$ for the immature specimens of *Haliotis* *gigantea* var. *discus* may probably be taken as an expression of a straight line. Consequently we may suppose NOMURA's view for young specimens to be true also in the case of the growth relation between the shorter and the longer diameters in *Haliotis gigantea* var. *discus*.

COMPARISON OF THE VALUES OF LOCAL RATIOS FROM TABLES 1 AND 2.

In Table 9 the numerical values of local ratios from Table 1 and from Table 2 are given together. It may be seen in this table that the value of the local ratio from Tables 1 and 2 shows a similar tendency to change with the difference of localities, but it is in general larger in Table 1 than in Table 2, due to the difference in methods of calculation. If we adopt the method which was applied in Table 1, we must be careful to collect a large number of specimens of the same size, because in the case of *Haliotis gigantea* var. *discus* the ratio between the shorter and the longer diameters ought to give different values according to size, that is, small specimens give a larger value, while larger specimens give a smaller value, but if we adopt the method which was applied in Table 2, we can collect specimens independent of size and of number. Thus I may infer that the latter method is more profitable than the former method in obtaining the local ratio, in determining species, in collecting specimens, and in the exactness of the results of investigation, even though there is some trouble while calculating.

TABLE 9.

	Omoi	Taro	Kosode	Ryori	Asamushi
Local ratio from Table 1	1.000	0.993	0.984	0.975	0.947
Local ratio from Table 2	1.000	0.990	0.986	0.969	0.937

THE INFLUENCE OF WATER TEMPERATURE ON THE GROWTH RELATION.

Food, waves (NOMURA, 1926a), physical and chemical properties of sea water, etc. may be factors causing the variation of shells, but

I could study only the effect of water temperature. Table 10 was prepared through the kindness of Mr. HOSOKAWA, who showed me the records from Aomori, and of Mr. KOIRO, who sent me the records from Todogasaki, Miyako, Kosode, and Hirota.

TABLE 10.

	Todogasaki (near Omoi) °C	Miyako (near Taro) °C	Kasode °C	Hirota (near Ryori) °C	Aomori (near Asamushi) °C
Jan.	a 8.53	a 7.47	e 6.00	a 6.87	a 6.99
Feb.	a 6.00	d 6.85	e 4.80	a 5.53	a 5.91
Mar.	a 5.40	d 3.05	e 4.70	b 5.45	a 6.62
Apr.	a 6.27	d 4.80	e 6.30	a 6.47	a 8.79
May	a 8.40	a 8.37	c 10.70	a 9.30	a 11.89
June	a 10.87	a 12.50	a 13.47	a 12.73	a 16.40
July	a 15.07	a 16.60	a 17.40	a 16.83	a 21.85
Aug.	a 18.57	a 20.47	e 19.10	a 20.03	a 24.36
Sept.	a 19.70	a 20.33	e 19.90	a 20.23	a 22.70
Oct.	a 17.53	a 17.17	f 16.50	a 17.13	a 18.18
Nov.	a 15.33	a 14.67	f 14.50	a 14.23	a 14.39
Dec.	a 11.83	a 12.30	f 9.50	a 9.40	a 9.67
Annual average	11.96	12.05	11.91	12.02	13.98

a mean temperature through 1918, 1919, and 1920.
b ,, ,, through 1918 and 1919.
c ,, ,, through 1919 and 1920.
d ,, ,, through 1918 and 1920.
e ,, ,, in 1918.
f ,, ,, in 1920.

Since the temperatures given in Table 10 are not from the exact localities where the specimens of *Haliotis gigantea* var. *discus* were collected and are surface temperatures, the data in this table may not be suitable in explaining the cause of variation of the shells. However, the annual average of temperature shows nearly the same degree at the localities of Iwate-Ken, while it is discordantly high at Asamushi; and as seen in Table 9 and in Plate 1, the degree of variation is comparatively small among the specimens from the localities of Iwate-Ken, while it is discordantly large in the specimens from Asamushi. From this fact it appears to me to be inferable that the variation of the shells may be effected by water temperature. If this consideration is allowed, we may conclude that, in general, a higher temperature produces a narrower shell in *Haliotis gigantea* var. *discus*.

THE GROWTH RELATION IN *Haliotis gigantea* AND IN *Haliotis gigantea* VAR. *mekai.*

At the close of the present report I wish to add some notes on the other varieties of *Haliotis gigantea,* though I had no chance of collecting and of directly investigating the specimens.

TABLE 11.

Haliotis gigantea Takanoshima

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
1	100.00	75.80	77.45	1.65
2	103.00	81.80	79.78	2.02
5	106.10	81.20	82.25	1.05
4	112.10	85.60	86.98	1.38
3	115.10	89.90	89.38	0.52
2	118.20	92.40	91.78	0.62
6	121.20	93.93	94.18	0.25
1	124.20	100.00	96.58	3.42
4	127.30	98.50	99.05	0.55
8	130.30	99.23	101.38	2.15
4	133.30	106.83	103.78	3.05
7	136.40	108.64	106.25	2.39
1	139.40	106.10	108.65	2.55
5	145.40	112.12	113.38	1.26
3	148.50	115.10	115.85	0.75
2	151.50	118.15	118.26	0.11
3	157.60	123.20	123.13	0.07
61				

TABLE 12.

Haliotis gigantea var. *mekai* Takanoshima

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
3	84.80	62.60	63.76	1.16
8	87.90	65.63	66.28	0.65
5	90.90	71.50	68.75	2.75
1	93.90	69.70	71.22	1.52
6	97.00	77.28	73.79	3.49
10	100.00	79.41	76.31	3.10
4	103.00	78.80	78.78	0.02
31	106.10	80.35	81.40	1.05
17	109.10	83.76	88.87	0.11
31	112.10	86.50	86.39	0.11

No. of specimens	Longer diameter	Shorter diameter		
		Observed	Calculated	Difference
27	115.10	89.44	88.91	0.53
18	118.20	90.23	91.53	1.30
40	121.20	94.24	94.10	0.14
25	124.20	96.36	96.62	0.26
1	125.70	93.90	97.88	3.98
26	127.30	99.07	99.24	0.17
1	128.80	103.00	100.50	2.50
30	130.30	101.73	101.81	0.08
16	133.30	104.18	104.33	0.15
18	136.40	107.75	107.00	0.75
2	139.40	113.65	109.57	4.08
3	142.40	113.10	112.14	0.96
2	145.40	116.65	114.71	1.94
5	151.50	118.14	119.95	1.81
2	154.50	116.65	122.57	5.92
2	157.60	125.75	125.24	0.51
1	160.60	124.20	127.86	3.66
1	163.60	127.30	130.44	3.14
331				

Fortunately the measurements of the shorter and the longer diameters of these varieties were taken (Tables 11 and 12) by OKAMURA, SENO, and HARA (1920) from the specimens from Takanoshima, and thus I could obtain the following relations:

In *Haliotis gigantea* $S=0.706L^{1.02}$

In *Haliotis gigantea* var. *mekai* $S=0.504L^{1.00}$

The shells of *Haliotis gigantea* and of *Haliotis gigantea* var. *mekai* can be distinguished from each other and from *Haliotis gigantea* var. *discus* by the convexity of the shell, in the position of the spire apex, etc.

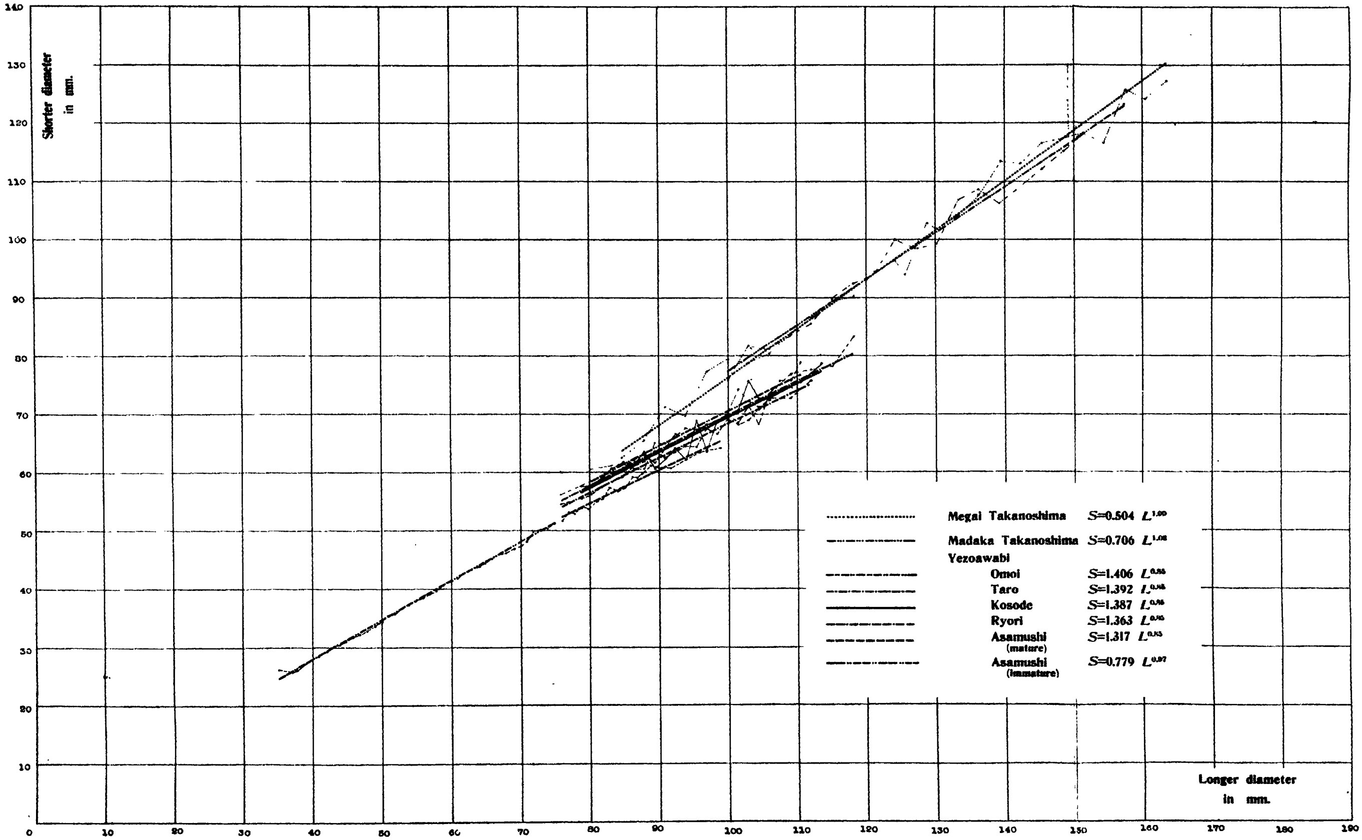
SUMMARY AND CONCLUSIONS.

1. The growth relation between the shorter and the longer diameters in *Haliotis gigantea*, *Haliotis gigantea* var. *mekai*, and *Haliotis gigantea* var. *discus* can be expressed by the equation $S=kL^x$, where S is the shorter diameter, L the longer diameter, k the local constant, and x the specific exponent.
2. The probable values of the specific exponent are as follows:

0.85 for mature specimens of *Haliotis gigantea* var. *discus*

0.97 for immature specimens of *Haliotis gigantea* var. *discus*

K. SASAKI. Growth relation in earshells.



The plate was plotted from Tables 2-5, 7, 8, 11, and 12. The thinner lines denote the relations between the shorter and the longer diameters obtained from the actual measurements, and the thicker lines denote the relations expressed by the equations. S shorter diameter, L longer diameter.

Report of the Biological Survey of Mutsu Bay.

1. General Outline of the Biological Survey of Mutsu Bay.*

By

SANJI HOZAWA.

(Biological Institute, Tohoku Imperial University, Sendai, Japan.)

Mutsu Bay covers 140°40′—141°15′E of longitude and 40°50′—41°17′N of latitude and lies at the extreme northern end of the main island of Japan, Hondo. From the middle of the coast Natsudomari Peninsula projects halfway into the bay, thus dividing it into two divisions: Noheji Bay on the east and Aomori Bay on the west. The distance between the east and the west coast is thirty-two miles, between the south and the north twenty-eight miles in the widest region and eight miles in the narrowest. The northwestern corner of the bay opens directly into Tsugaru Strait and through this opening faces Hokkaido Island.

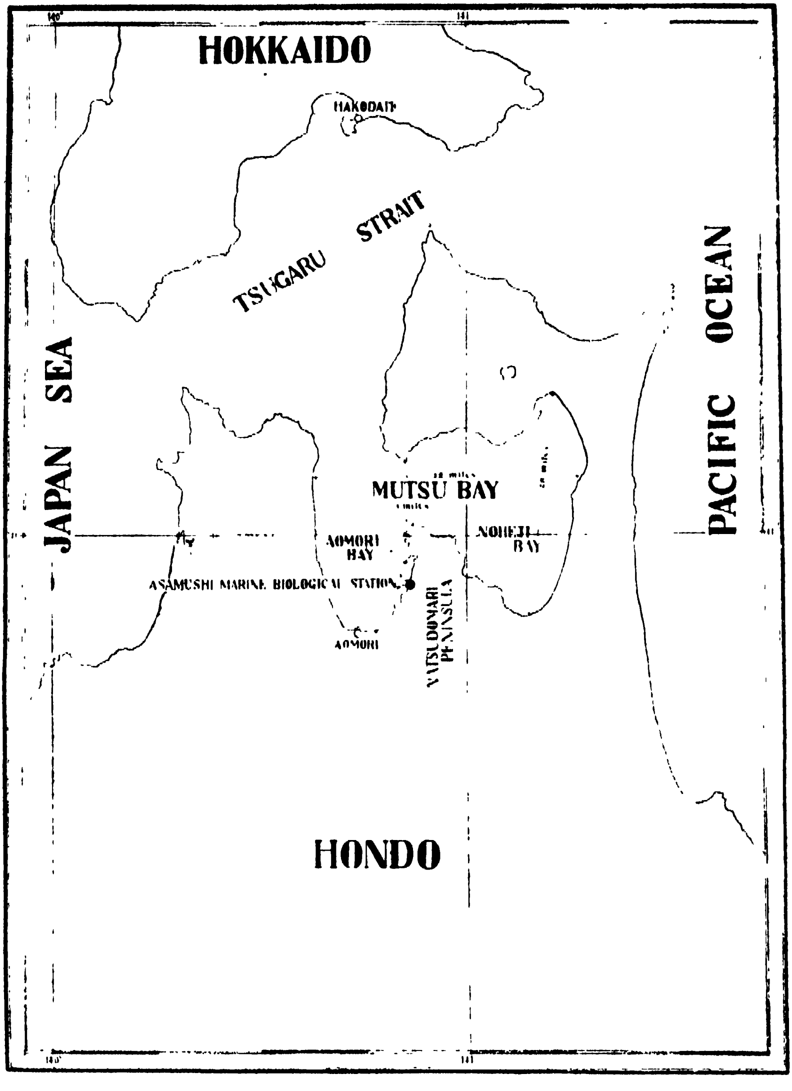
In July of 1924 the Tohoku Imperial Univesity established the Marine Biological Station in the neighborhood of Asamushi, which is located along the eastern coast of Aomori Bay. The number of investigators who visit the station increases annually at a rapid rate and the demand for materials for investigation naturally increases equally rapidly. However, we were often embarrassed about supplying adequately the amount and kinds of materials for those zealous research workers on account of our imperfect knowledge concerning the fauna and flora in this great bay. For this reason the biological survey of this bay became very important.

Although our immediate object of this survey was thus to facilitate the work of the investigators, at the same time we had several other reasons, some of which are stated below:

1) Since the bay has a narrow outlet to the bigger sea the survey can be accomplished relatively easily and concisely.

---

* A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.



Map of Mutsu Bay.

2) On account of the location of the marine biological station the survey can be greatly facilitated and accomplished with less expense.

3) Since Mutsu Bay is in contact with both the Pacific Ocean and the Japan Sea through Tsugaru Strait an adequate knowledge concerning the distribution of the fauna and flora may bring out some important data in the field of bio-geography.

4) Inasmuch as it is our long standing wish to make a similar survey of the entire northeastern coast of Japan, the present small undertaking now in progress will give us valuable experience for such future work on a greater scale.

The present biological survey of Mutsu Bay was made possible by an adequate financial grant given by the Saito Ho-on Kai (The Saito Gratitude Foundation) which was established by the late ZENEMON SAITO Esq. three years ago for the promotion of scientific research and for social and industrial improvement. Taking this opportunity, we wish to acknowledge our deep obligation to the Foundation for this generous support.

This survey was begun last April and was carried on actively till the end of September. In carrying out the survey we divided the work into three large divisions and divided our responsibilities respectively:

Professor SANJI HOZAWA (Tohoku Imperial University) in charge of fauna,

Professor MASATO TAHARA (Tohoku Imperial University) in charge of flora,

Assistant Professor SEIJI KOKUBO (Asamushi Marine Biological Station) in charge of plankton organisms.

It was agreed that each be entirely responsible for his respective field, as, for instance, in the collection of specimens, the choice of collection stations, the distribution of materials to the specialists, etc. It is also intented to publish fuller reports concerning the survey in general in the near future.

The number of specimens thus far collected is quite large and it is self evident that only by the cooperation of many specialists the difficult task of identification of known species, of description of new species, etc. or, in short, the object of the present undertaking, can be accomplished. In this connection we are very happy to mention that biological friends showed a great interest and willingly accepted our wish in cooperating actively.

With deepest appreciation and gratification we announce the names of collaborators together with their field of responsibilities:

TORU ABE, Rigakushi, Tokyo Imperial University. *Protozoa.*

SEITARO GOTO, Rigakuhakushi, Tokyo Imperial University. *Crinoidea, Echinoidea, Asteroidea.*

SHOGO HARUYAMA, Rigakushi, Kyoto Imperial University. *Cirripedia.*

TOYOJI HIKIDA, Hokkaido Imperial University. *Pisces.*

SANJI HOZAWA, Rigakuhakushi, Tohoku Imperial University. *Porifera.*

AKIRA IZUKA, Rigakuhakushi, The Peers' School, Tokyo. *Polychaeta.*

NAGAMICHI KURODA, Rigakuhakushi, Tokyo. *Aves.*

HIKOSHICHIRO MATSUMOTO, Rigakuhakushi, Tohoku Imperial University. *Ophiuroidea.*

G. E. NICHOLLS, University of Western Australia. *Amphipoda, Isopoda.*

ASAJIRO OKA, Rigakuhakushi, Tokyo Higher Normal School. *Tunicata.*

YAICHIRO OKADA, Tokyo Higher Normal School. *Polyzoa, Chaetognatha.*

HIROSHI OSHIMA, Rigakuhakushi, Kyushu Imperial University. *Holothurioidea.*

MADOKA SASAKI, Rigakuhakushi, Hokkaido Imperial University. *Cephalopoda.*

SHUNICHI TAKATSUKI, Rigakushi, Asamushi Marine Biological Station of the Tohoku Imperial University. *Gastropoda, Lamellibranchiata, Scaphopoda.*

TORU UCHIDA, Rigakushi, Tokyo Imperial University. *Cœlenterata.*

YU YOKOYA, Suisangakushi, Tokyo Imperial University. *Brachiura.*

We trust that before long the materials which are not yet assigned will be also in the hands of specialists.

In preparing the reports it was agreed by all investigators concerned that even species well known to science, if they are important material for general research purpose and if, at the same time access to the original papers is difficult, may be fully described or even illustrated in colors. We may also add that as far as possible the reports will appear in the present journal "The Science Reports, Series IV," whenever they are ready to be printed, under one common title, "Report of the Biological Survey of Mutsu Bay", accompanied by a subtitle which indicates the nature of the particular research by each investigator on whom the entire responsibility for the given report rests.

We feel that it is our pleasant duty to make a special acknowledgement here for our appreciation to the following gentlemen: to Professor SEITARO GOTO of Tokyo Imperial University and to Professor AKIRA IZUKA of the Peers' School for valuable advise given in making out a general plan and for active scientific cooperation from the beginning of the enterprise, and to Professor SHINKISHI HATAI, Director of the Asamushi Marine Biological Station, in permitting us to use the facilities of the entire laboratory.

Finally, we wish to add that this biological survey of Mutsu Bay will be continued in the future till the object is adequately accomplished.

December 8, 1926.

Reprot of the Biological Survey of Mutsu Bay.

2. Medusae of Mutsu Bay.*

By

TOHRU UCHIDA.

(Zoological Institute, Science Faculty, Imperial University, Tokyo.)

This is a report on medusae collected in Mutsu Bay during the three years 1924–1926. Numerous specimens examined by me include 22 species, namely, 14 belonging to the Hydromedusae, 6 to the Scyphomedusae, and 2 to the Ctenophorae. Of these, one medusa is uncertain and two others have been newly recorded from the country; one is *Melicertum*, which is common on the northern Atlantic coast of America and the other is a new variety of *Rhopilema esculenta.*

The medusae found in the bay are as follows.

Hydromedusae.

Ord. Anthomedusae.

1) *Euphysa* sp.
2) *Hybocodon prolifer* L. AGASSIZ.
3) *Cladonema radiatum* var. *mayeri* PERKINS.
4) *Urashimea globosa* KISHINOUYE.
5) *Turritopsis nutricula* McCRADY.
6) *Rathkea blumenbachii* (RATHKE).
7) *Spirocodon saltatrix* (TILESIUS).

Ord. Leptomedusae.

8) *Melicertum campanula* L. AGASSIZ.
9) *Obelia* sp.
10) *Phialidium discoida* (MAYER).
11) *Aequorea coerulescens* (BRANDT).
12) *Gonionema murbachi* var. *oshoro* UCHIDA.

Ord. Trachomedusae.

---

* A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

13) *Aglantha digitale* HAECKEL.
14) *Liriope tetraphylla* (CHAMISSO et EYSENHARDT).

Scyphomedusae.

Ord. Cubomedusae.
15) *Charybdea rastonii* HAACKE.

Ord. Stauromedusae.
16) *Haliclystus auricula* CLARK.
17) *Sasakiella cruciformis* OKUBO.

Ord. Saemostomae.
18) *Dactylometra pacifica* GOETTE.
19) *Cyanea capillata* ESCHSCHOLTZ.

Ord. Rhizostomae.
20) *Rhopilema esculenta* var. *asamushi*, n. var.

Ctenophorae.

21) *Hormiphora palmata* CHUN.
22) *Beroë ramosa* KOMAI.

From the viewpoint of oceanographical distribution, the fauna in Mutsu Bay is very interesting. There are found many arctic species, such as *Hybocodon prolifer*, *Urashimea globosa*, *Rathkea blumenbachii*, *Melicertum campanula*, *Gonionema murbachi* var. *oshoro*, *Aglantha digitale*, *Haliclystus auricula*, *Sasakiella cruciformis*, *Cyanea capillata*, and *Beroë ramosa*. These medusae, except *Urashimea globosa*, *Rathkea blumenbachii*, and *Haliclystus auricula*, have never been found on the Pacific coast of Honshu except in Mutsu Bay. Therefore, the fauna is, as a whole, similar to that of Oshoro, Hokkaido. On the other hand, the following three medusae, viz. *Spirocodon saltatrix*, *Aequorea coerulescens*, and *Dactylometra pacifica*, which are very common in the water of Honshu and Kiushu, have never been recorded from Hokkaido, as far as I know. Pelagic medusae, which are occasionally found swarming in the bay, are *Hybocodon prolifer*, *Rathkea blumenbachii*, *Aequorea coerulescens*, and *Aglantha digitale*, but large medusae, such as *Spirocodon saltatrix* and Scyphomedusae seem to be found only in small numbers. On *Sargassum*, there are commonly found four medusae, such as *Cladonema radiatum* var. *mayeri*, *Gonionema murbachi* var. *oshoro*, *Haliclystus auricula*, and *Sasakiella cruciformis* during July and August, but they disappear when the sea-weeds die.

Finally, I should like to express here warm thanks to Prof. S. HATAI and Prof. S. HOZAWA, who were so kind in helping my study in various ways, and also to Assist. Prof. S. KOKUBO and Mr. S. TAKATSUKI for collecting materials.

Hydromedusae.

ORD. ANTHOMEDUSAE.

1) **Euphysa** sp.

The bell is 1 mm. high and 0.8 mm. wide. The gelatinous substance is uniformly thin. Four radial canals, straight and equal in length. A ring canal rather broad. Tentacle bulbs four in number and very large. An ocellus on the protruded outer side of the bulb. Tentacles four in number and filamentar, all provided with many ring-like nematocyst clusters. Of the four tentacles, one is by far the longest, the two adjacent to it, intermediate, and the one opposite it, the smallest. They are all hollow. Velum well-developed. Manubrium somewhat cone-like. Gonads are not ripe in the specimen.

Colour. Ocelli light red. Other parts colourless in the preserved specimen.

Loc. Asamushi, March 14, 1926.

Remarks. The medusa must be referable to a new species because of the presence of ocelli and the structure of tentacles, but as there is only one specimen the specific name will be reserved for further study.

2) **Hybocodon prolifer** L. AGASSIZ.

Hybocodon prolifer; AGASSIZ, L., 1862, Cont. Nat. Hist. U. S., vol. 4, p. 243 et 343, pl. 23a, fig. 10, 11, pl. 25, fig. 1-19. UCHIDA, 1925, Jap. Jour. Zool., vol. 1, p. 78, fig. 1.

A large number of the medusa were collected by Assist. Prof. S. KOKUBO and Mr. TAKATSUKI by a surface tow in the spring. In most of the specimens, both sexual and asexual reproduction occur simultaneously, as described in the American medusa. The species is very common in the arctic region of both the Atlantic and the Pacific.

Loc. Asamushi, March 14, 1926.

3) **Cladonema radiatum** var. **mayeri** PERKINS.

Cladonema mayeri: PERKINS, 1906, Year Book of the Carnegie Inst. of Washington, no. 4, p. 118.

Cladonema radiatum var. *mayeri:* UCHIDA, 1925, Jap. Jour. Zool., vol. 1, p. 81, fig. 7.

The medusa is commonly found attached on *Sargassum* in the bay during July and August. All specimens examined by me agree with the species formerly recorded (1925) in the presence of the regular number of tentacles (9), gonads (6), and oval tentacles (6). The small tentacles, which were described as suckers, are undoubtedly young filamentar ones, as suggested in my last paper. For the first time, the tip of the short, young tentacles is covered with nematocysts and subsequently the dorsal side is gradually bordered by several series of nematocyst batteries, regularly arranged and developed from the terminal to the basal portion, so nematocyst batteries are always more prominent on the dorsal side of tentacles than on the ventral side. Out of the specimens obtained in the bay, the largest one is 3.5 mm. high and 3 mm. wide.

Loc. Yunoshima, July 13, 1926. Namiuchi, Hirauchimura, July 17, 1926. Tsuchiya, July 14, August 5, 1925 and July 2, 1926.

4) **Urashimea globosa** KISHINOUYE.

Urashimea globosa; KISHINOUYE, 1910, Jour. Coll. Sci. Imp. Univ. Tokyo, vol. 27, art. 9, p. 28, pl. 5, fig. 27–29. MAYER, 1910, Medusae of the world. vol. 3. p. 722. HARTLAUB, 1913, Nordisches Plankton, Bd. 12, p. 344, Fig. 290–292.

Urashimea macrotentaculata; KISHINOUYE, 1910, Jour. Coll. Sci. Imp. Univ. Tokyo, vol. 27, art. 9, p. 29, pl. 5. fig. 30.

Zanclea maasi; UCHIDA, 1925, Jap. Jour. Zool. vol. 1, p. 80, fig. 2–5.

Zanclea sp.; MAAS, 1909, Abhandl. K. Bayer. Akad. Wiss. Suppl Bd. 1, Abhandl. 8, p. 8, Taf. 1, Fig. 2. HARTLAUB, 1914, Nordisches Plankton, Bd. 12, p. 400.

Several specimens of this interesting medusa were caught by Prof. S. HOZAWA and Mr. TAKATSUKI. Out of specimens examined by me, the largest one is 11 mm. high.

Recently several specimens of the medusa were sent through the kindness of Prof. A. OKA. They are in different stages and represent the structures of *Zanclea maasi*, *Urashimea globosa*, and *Urashimea macrotentaculata* respectively, so I wish to condense these specific names into one. The metamorphosis of the species will be given in another paper.

Loc. Okunai, July 8, 1926.

5) **Turritopsis nutricula** McCRADY.

Turritopsis nutricula; McCRADY, 1857, Gymn. Charleston Harvor, p 25, pl. 8, fig. 1. UCHIDA, 1925, Jap. Jour. Zool, vol. 1, p. 84, fig. 8-9.

Four young specimens belonging to the common species were obtained. They are about 1 mm. high, and are provided with 24 tentacles and four simple lips. Gonads are not yet developed.

Loc. Asamushi, March 14, 1924.

6) **Rathkea blumenbachi** (RATHKE).

Rathkea blumenbachi: UCHIDA, 1925, Jap. Jour. Zool., vol. 1, p. 86.

Rathkea octopunctata var. *grata;* MAYER, 1910, Medusae of the World, vol. 1, p. 179, pl. 18, fig. 5.

Lizzia shimiko; KISHINOUYE, 1910, Jour. Coll. Sci., Imp. Univ. Tokyo, vol. 27, art. 9, p. 25, pl. 5, fig. 24.

Oceania blumenbachic; RATHKE, 1835, Mém. Acad. Imp. Sci. St. Pétersbourg, tome 2, p. 321, pl. 1, fig. 1-4.

A great number of the common medusa were obtained by Assist. Prof. S. KOKUBO at Asamushi. They are in different stages of development and give rise to medusa buds on their manubrium. As the species has been described several times, the specific diagnosis is omitted here.

Loc. Asamushi, March 14, 1926.

7) **Spirocodon saltatrix** (TILESIUS).

Spirocodon saltatrix; MAAS, 1909, Abhandl. K. Bayer. Akad. Wiss. Suppl.-Bd. 1, Abhandl. 8, p. 18, Pl. 2, Fig. 10-13. MAYER, 1910, Medusae of the world, vol. 1, p. 220, fig. 112, —, 1910, ditto, vol. 3, p. 724. OKADA, 1926, Ann. Zool. Jap., vol. 11, p. 76-79 et 81-85, fig. 1-4, pl. 1, fig. 1-2.

Spirocodon brevitentacularis (moribund medusa); OKADA, 1926, Ann. Zool. Jap., vol. 11, p. 80–81, pl. 1, fig. 3–4.

Goneomeandrus chrysostephanus; KIRKPATRICK, 1903, Ann. Mag. Nat. Hist., vol. 12. p. 616, pl. 33, fig. 1–4.

Medusa saltatrix; TILESIUS, 1818, Mém. Acad. St. Pétersbourg, tome 6, p.554, pl. 18.

Several specimens were obtained in the neighbourhood of the Asamushi Marine Biological Station. All specimens examined by me were by far larger than those ever caught in other localities. They were about 70 mm. high and 65 mm. wide and were provided with about 60 hollow tentacles in each octant. As far as my observations go, the medusa seems to be referred to the Anthomedusae. The discussion on the systematic position of the species will be given in another paper.

Loc. Namiuchi, Moura, and Shimanai, March 10 and May 5, 1925.

ORD. LEPTOMEDUSAE.

8) **Melicertum campanula** L. AGASSIZ.

Melicertum campanula; AGASSIZ, L., 1862, Cont. Nat. Hist. U. S., vol. 4, p. 349 et 352. MAYER, 1910, Medusae of the world, vol. 1, p. 207, pl. 23, fig. 4-5, pl. 24, fig. 5.

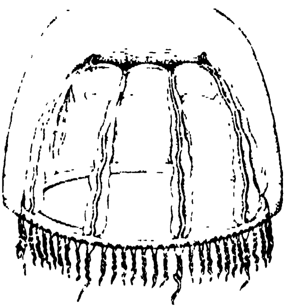


Fig. 1. *Melicertum campanula.* 7 mm. high.

Bell higher than a hemisphere and flat-topped, 7 mm. high and 8 mm. wide. The gelatinous substance is fairly thick, especially at the aboral pole. There are eight simple, broad radial canals, which are equally developed and which become wider at the basal portion. They are somewhat undulating, probably on account of shrinkage. Tentacles 64 in number, 8 in each octant. These tentacles, arise from the bell margin, and each has a large bulb at its base. They are tapering and coiling, hollow and short. The ocelli could not be seen in preserved specimens. There are no sensory clubs or statocysts between the tentacles. The velum is stout but narrow. The manubrium is wide, shallow, and provided with eight recurved lips, which correspond to the eight radial canals. There are eight gonads, which cover the entire length of the eight radial canals. They are especially developed in the lower portion, showing several foldings, as seen in gonads of *Ptychogena*.

Colour. According to Mr. TAKATSUKI, who obtained two specimens of the species, radial canals and manubrium were white when living. In the preserved specimens, gonads and lips are slightly brown.

Loc. Tsuchiya, June 5, 1926.

Remarks. There are known two certain species in the genus: *Melicertum campanula* and *M. octocostatum*, both occurring in the Atlantic. The two species above mentioned are pyriform in shape and are provided with a well-developed apical solid gelatinous substance, while the Japanese specimens are flat-topped in shape. According to MAYER's figures (1910), however, the young medusa of *M. campanula* has no apical jelly mass, so our specimens, of smaller size than the former, may be referrable to *Melicertum campanula*. On the other hand, any indication of the several longitudinal lines of nematocyst-like cells, which are present on the subumbrella of *Melicertum octocostatum*, could not be found in the Japanese medusae.

9) **Obelia** sp.

Several specimens were obtained by a surface tow on August 25, 1926. The medusa is 1.5 mm. in diameter and is provided with 18 tentacles in each quadrant, arranged younger and older alternating. Gonads are situated on the radial canals, a little nearer the marginal portion. The species is absolutely colourless. It is difficult for us to identify medusae belonging to the genus without any knowledge of their hydroid.

10) **Phialidium discoida** (MAYER).

Phialidium discoida; BIGELOW, 1909, Mem. Mus. Comp. Zool., Harvard coll., vol. 37, p. 155, pl. 6, fig. 8, pl. 38, fig. 6-7. MAAS, 1909, Abhandl. d. math.-phys. Klass. d. K. Bayer. Akad. d. Wissen., Suppl.-Bd. 1, Abhandl. 8, p. 25. MAYER, 1910, Medusae of the World, vol. 2, p. 272. UCHIDA, 1925, Jap. Jour. Zool., vol. 1, p. 90, fig. 14.

Oceania discoida; MAYER, 1900, Bull. Mus. Comp. Zool. at Harvard coll., vol. 37, p. 51, pl. 20, fig. 53-55.

Several young specimens belonging to the species were obtained in the bay on Sept. 16, 1923. The youngest medusa, with a diameter of 2 mm., has four perradial tentacles and the same number of interradial ones, the latter being smaller than the former. The tentacles consist of a prominent tentacle bulb and a narrow filamentous portion which is tightly coiled. Besides these eight tentacles, the medusa has five tentacle-bulbs, each being situated in one octant between the perradial and the interradial tentacles, while in three octants no indication of them could be found. Four gonads, round in shape, at the basal portion of the four radial canals. Statocysts alternate with tentacles in position. Manubrium short.

A larger medusa, with a diameter of 4 mm., has three short tentacles between the perradial ones. Two statocysts are present between two tentacles. Short manubrium squarish and brown gonads well-developed, situated close to the ring canal. The velum is narrow.

Loc. Asamushi, Sept. 16, 1924.

Remarks. The species was at first recorded by MAAS (1909) from Japan and recently by me (1925) from Oshoro.

11) **Aequorea coerulescens** (BRANDT).

Aequorea coerulescens; TORREY, 1909, Univ. California Publ. Zool., vol. 6, No. 2, p. 28. BIGELOW, 1909, Mem. Mus. Comp. Zool., Harvard Coll., vol. 37, p. 177, pl. 4, fig. 4 and pl. 35, fig. 3–8.

Aequorea forskalea (in part): MAYER, 1910, Medusae of the World, vol. 2, p. 325.

Mesonema (Zygodactyla) coerulescens; BRANDT, 1838, Mém. Acad. Imp. Sci. St. Pétersbourg, sér. 6, Sci. Math. et Nat., tome 4, p. 360, pl. 5.

Mesonema pensile; MAAS, 1909, Abhandl. math.-phys, d. Klass. d. K. Bayer. Akad. d. wissen., Suppl.-Bd. 1, Abhandl. 8, p. 26.

Zygodactyla coerulescens; BRANDT, 1838, Mém. Acad. Imp. Sci. St. Pétersbourg, sér. 6, tome 4, p. 360, pl. 5.

The bell is very flat and discoid, 134 mm. in diameter. The gelatinous substance is soft and massive, as in the saemostome medusa *Aurelia*, but especially thick in the aboral portion of the stomach, measuring 20 mm. and gradually becoming thinner towards the peripheral margin. The margin is recurved inwards, as in *Aurelia*. The recurved portion is 11 mm. in width and becomes wider as the medusa grows.

The diameter of the stomach and mouth (without lips) is 65 mm. and 42 mm. respectively. Numerous radial canals, having almost equal intervals, arise separately from the edge of the stomach and extend straight out to the ring canal. The radial canals, 85 in number, are straight and are arranged so that a young and narrow one comes between older and wider ones. The tentacles are numerous and are equally developed, arranged in two rows, the younger on the inner side. In the swimming medusa they become filamentar, and are very long, but in preserved specimens, they are short, tapering, and coiled. The tentacles are hollow and are covered with ring-like nematocyst clusters. The exeretory papillae are present only on the axial base of well developed tentacles. There are numerous statocysts between the tentacles. They can not be seen without difficulty on account of the latter. The velum is narrow but stout. The manubrium is very flat, its ventral wall has numerous meridional ridges which almost correspond to the number of the radial canals, but which are, strictly speaking, a little fewer. The ridges lead to the terminal end of each lip, interferring with the lines of the ring muscles, which are arranged concentrically on the wall of the manubrium. The peripheral wall of the stomach is somewhat folded and bulges out. The lips are 60 in number and are tapering, with folded margins showing the same structure as the oral arms in *Aurelia.* The narrow linear gonads are developed upon these canals and cover the whole length of the radial canals except the terminal portion close to the ring canal. They are at first linear but gradually become folded, and the middle portion is the widest.

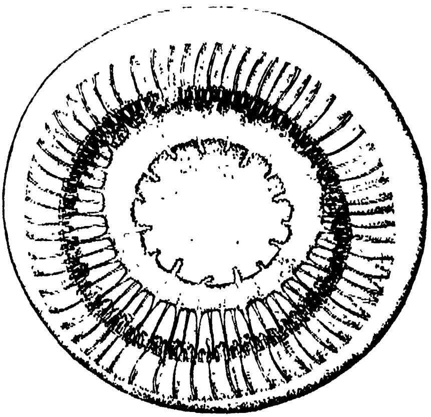


Fig. 2. *Aequorea coerulescens.*

Colour. Tentacles deep blue. Radial canals and lips blue. Gelatinous substance bluish.

Loc. Off Yunoshima, May 1926.

Remarks. Several specimens were caught in the bay by Mr.

TAKATSUKI. In the medusae, the number of radial canals and lips do not correspond with the diameter of the umbrella as shown in the following table.

	Spec. A	Spec. B	Spec. C	Spec. D
Diameter of umbrella.	25 mm.	31 mm.	132 mm.	134 mm.
Diameter of stomach.	14 mm.	16 mm.	73 mm.	68 mm.
Number of radial canals.	60	61	108	85
Number of lips.	16	20	57	66
Thickness of gelatinous substance.	8 mm.	10 mm.	20 mm.	20 mm.

Moreover, the time of development of the gonads is variable according to individuals.

The medusa bears some resemblance to *Aequorea forskali* and *A. pensile*, but is easily distinguished by the fewer number of radial canals and the considerably numerous tentacles, which are several times as many as the radial canals. MAAS (1909) recorded as *M. pensile* a medusa, from Sagami Bay, belonging to the genus. His specimens were broken and had no marginal tentacles, so the identification was based upon the number of radial canals, and the diameters of the umbrella and the stomach. But the relative number of these organs can not be utilized in specific identification, as mentioned above. Though the specimens in question are provided with more radial canals, they are probably the same as my species, as I have examined several specimens of *A. coerulescens* collected at Misaki.

12) **Gonionema murbachi** var. **oshoro** UCHIDA.

Gonionema murbachi var. *oshoro*; UCHIDA, 1925, Jap. Jour. Zool., vol. 1, p. 84, fig. 18–19.

A great number of living or preserved medusa belonging to the species were examined by me. The well-developed medusa is very flat in shape as described in my last paper, but the youngest medusa among them is 3 mm. wide and 2 mm. high and is provided with six tentacles in each quadrant. Gonads make their appearances on the uppermost portion of four radial canals in the medusa. As the medusa grows, the bell becomes gradually flatter and finally takes a quite different shape in a later stage. It is also noticeable that the medusa is liable to have a higher bell in a preservative than in the living state. The species is very common and attaches on *Sargassum* at Asamushi as well as in Oshoro.

Loc. Yunoshima, July 13, 1926. Namiuchi, Hirauchimura, July 17, 1926. Moura, Hirauchimura, July 20, 1926. Tsuchiya, July 3, 1926.

ORD. TRACHOMEDUSAE.

13) **Aglantha digitale** HAECKEL.

Aglantha digitalis; HAECKEL, 1879, Syst der Medusen, p. 272, Tab. 16, Fig. 5, 6.

Aglantha digitale; BIGELOW, 1913, Proc. U. S. Nat. Mus., vol. 44, p. 43. UCHIDA, 1925, Jap. Jour. Zool., vol. 1, p. 97.

Several medusae belonging to the common arctic species were collected among innumerable diatoms. Out of specimens examined by me, the smallest one is 5 mm. high and 3.2 mm. wide. Eight gonads make their appearances as a round sac on the uppermost part of eight radial canals. The largest specimen is 12 mm. high and 7 mm. wide. Gonads develop as eight sausage-like masses, hanging down from the upper part of radial canals into the bell cavity. The medusa is occasionally tinted pinkish.

A young medusa was already from Oshoro in 1925.

Loc. Asamushi, March 10, 1925.

14) **Liriope tetraphylla** (CHAMISSO et EYSENHARDT).

Liriope tetraphylla; BIGELOW, 1913, Proc. U. S. Nat. Mus., vol. 44, p. 55.

Liriope rosacea; MAAS, 1909, Abhandl. d. math.-phys. Klass. d. K. Bayer. Akad. d. Wissen. Suppl.-Bd. 1, Abhandl. 8, p. 31.

Geryonia tetraphylla; CHAMISSO et EYSENHARDT, 1821, Nova Acta Phys. Med. Acad. Leop. Carol., tome 10, p. 357, pl. 27, fig. 2.

Several young specimen were examined. As they were all in a young stage, definite identification could not be made, but they are probably to be referred to the species, which is very common in any Japanese water.

Loc. Asamushi, Sept. 16, 1924. Yunoshima, Sept. 30, 1926. Hadakaiwa, Oct. 16, 1926.

Scyphomedusae.

ORD. CUBOMEDUSAE.

15) **Charybdea rastonii** HAACKE.

Charybdea rastonii; HAACKE, 1887, Jen. Zeit. f. Naturw., Bd. 20, p. 591, Pl. 35, Fig. 1–15. MAYER, 1906, Bull. U. S. Fish. Commission, vol. 23, pt. 3, p. 1134, pl. 1, fig. 1–1 c. BIGELOW, H. B., 1909, Mem. Mus. Comp. Zool. at Harvard Coll., vol, 37, p. 17, pl. 1, 10. MAAS, 1909, Abhandl. d. math.-phys. Klas. d. K. Bayer. Akad. d. Wissen. Suppl.-Bd. 1, Abhandl. 8, p. 41. MAYER, 1910, Medusae of the world, vol. 3, p. 508, 726. —, 1913, Pub. Carnegie Inst. Wash., no. 214, p. 170. YATSU, 1913, Journ. Coll. Sci., Tokyo, vol. 40, art. 3, p. 1. —, Dobutsugaku Zassi, vol. 30, p. 24. MAYER, 1917, Smithonian Inst. Nat. Mus., Bull. 100, vol. 1, pt. 3, p. 187. STIASNY, 1912, Vid. Medd. f. Dansk naturh. Foren Bd. 73, p. 515.

Charybdea brevipedalia; KISHINOUYE, 1891, Dobutsugaku Zassi, vol. 3, p. 437, fig. 1–3.

Charybdea (young); UCHIDA, 1926, Proc. Imp. Acad., vol. 2, p. 295, fig. 1.

Two fine specimens of the species were examined by me. The larger one is 35 mm. high and 32 mm. wide. The medusa is common during August and September in Japanese waters. It is not unworthy of mention that those found in the Japan Sea and in Hokkaido are generally larger than those in the Pacific but no other remarkable difference could be seen. Recently a young medusa with a height of 1.2 mm. was recorded.

Loc. Hadakaiwa, Oct. 5, 1926.

ORD. STAUROMEDUSAE.

16) **Haliclystus auricula** CLARK.

Haliclystus auricula; CLARK, H. J., 1863, Jour. Boston Soc. Nat. Hist., vol. 7, p, 559. MAYER, 1910, Medusae of the World, vol. 3, p. 532.

Haliclystus tenuis; KISHINOUYE, 1910, Jour. Coll. Sci., Imp. Univ., Tokyo, vol. 27, art. 9, p. 4, pl. 1, fig. 3.

The bell, without the peduncle, is octangular-pyramidal, gradually expanding towards the bell margin, 9 mm. high and 10 mm. wide. The peduncle is quadrate and with four interradial furrows, about 6 mm. long and 1.7 mm. in diameter, and gradually widing towards the basal disc. The whole surface of the animal is finely granulated with nematocysts. The gelatinous substance is thin and rigid. The marginal portion of the bell is divided into 8 parts, making eight arms, one in each adradius. Each arm is provided with a tentacle cluster. The four perradial clefts between the tentacle clusters are deeper than the four interradial ones. Each tentacle cluster consists of about 30–40 knobbed tentacles, which are hollow, and are provided with globular knobs, which are covered with nematocysts, at their terminal ends. They are about 0.8 mm. in length. The eight marginal anchors, which are situated in each perradius and interradius, are kidney-shaped and are mounted upon a short cylindrical peduncle, the perradial four among them being situated at a higher level than the interradial four. Their diameter is about half that of the peduncle. The manubrium is short and four sided, and liable to be folded on account of its thinness. The gastric filaments are numerous in each perradius, arranged along about the lower half part of the gastric cavity. There are eight adradial gonads, making four pairs in each perradius. These gonads are narrow, extending to the ends of the eight arms from the aboral end of the umbrella, each consisting of about forty or more out-folded vecicles, arranged in about two rows. These vecicles are various in size according to the stage. The peduncle has four interradial tracts of longitudinal muscle-fibers and four perradial canals, which communicate with the central stomach cavity of the umbrella.

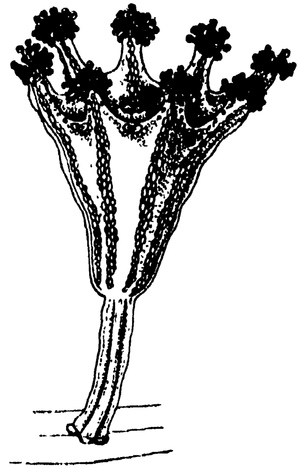


Fig. 3. *Haliclystus auricula.* 17 mm. high.

Colour. Colour very variable; umbrella brown or green with scattered white spots, which are especially conspicuous on the bell margin. The peduncle is generally light brown or green. The knobs of the marginal tentacle are brown, and the stem is green. The adradial parts of the medusa are brown. The marginal anchors are bright brown and the gonads brown or deep port wine coloured.

Loc. Tsuchiya, July 27, 1926. Zizo, July 2, 1926.

Remarks. The medusa is very common on *Sargassum* from the beginning of July to the end of August. A young medusa was recorded from Oshoro by KISHINOUYE (1910) as *Haliclystus tenuis*. According to his description, this medusa has eight genital glands, each consisting of many round sacs arranged in two longitudinal rows and a comparatively higher bell than that of medusae formerly recorded. Off course, the Japanese medusa is somewhat smaller in size and has fewer gonads, but I examined several specimens which were collected at Oshoro and the genital vecicles in each gonad are arranged in four rows. Moreover, the shape of the preserved medusa is more or less variable according to shrinkage, so the medusa in question seems to be a young one of *Haliclystus auricula*, which is commonly found in the northern Atlantic.

17) **Sasakiella cruciformis** OKUBO.

Sasakiella cruciformis; OKUBO, 1917, Dobutsugaku Zassi, vol. 29, p. 317, fig. 1–2.

Bell provided with four interradial arms, which are divided into two short, paired arms, therefore giving a nearly cruciform shape. Bell 10 mm. in diameter and 2.5 mm. in thickness, when expanded. Peduncle 3 mm. in length, becoming wider towards the basal end, which represents a adhesive disc. The gelatinous substance is transparent, with numerous small white spots, which are especially abundant in the perradial part of the subumbrella. The exumbrella is generally smooth, while the subumbrella has four interradial furrows, which begin at the base of the manubrium, leading to the marginal portion of the subumbrella. The primary tentacles are hollow and are eight in number, four in each perradius and another four in each interradius. These tentacles are tapering and each is provided with an adhesive disc at its terminal end and a white spot at the middle length. The perradial four are about 3 mm. long, while the interradial four 2 mm. The interradial arms become generally narrow towards

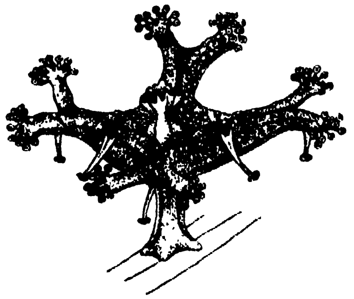


Fig. 4. *Sasakiella cruciformis.* 10 mm. in diameter.

the distal end, and are consequently divided into two short arms about 1 mm. long and which are provided with 10 hollow secondary tentacles at their ends. These tentacles are capped and are arranged in three radial rows, the outer three tentacles, which are older, being furnished with a white spot on their abaxial side. The manubrium has four thin lips, its perradial ridges each connecting with a perradial portion of the subumbrella, which is elevated. Gastral filaments make groups in each interradial part, close to the mouth. Gonads represent eight adradial rows of 9 succules, which extend from the base of the manubrium to the tip of the arms.

Colour. Exumbrella generally transparent, with small white spots, the entoderm brown and especially deeper brown along the marginal portion. Subumbrella also brown with white spots on the margin and the perradial parts. Stem, gelatinous substance, transparent, with some small white spots, its entoderm brown, especially deeper at the base. Manubrium light brown. The primary tentacles are white in the stem, slightly brown in the adhesive disc, and brownish purple at the base. Secondary tentacles brown.

Loc. Tsuchiya, Sept. 27, 1926.

Remarks. This interesting species was first recorded by OKUBO (1917) from Oshoro, Hokkaido. From the result of my investigation on the medusa at Oshoro last year and at Asamushi this year, those at Oshoro were blacker than those at Asamushi. However, as far as my observations go, medusae belonging to the order are generally considerably variable in colour. The remarkable characteristics in the genus lie in the primary tentacles, which are not metamorphosed into anchors.

The medusa is commonly found attached on *Sargassum.*

ORD. SAEMOSTOMAE.

18) **Dactylometra pacifica** GOETTE.

Dactylometra pacifica; BIGELOW, 1913, Proc. U. S. Nat. Mus., vol. 44, p. 92.

Dactylometra quinquecirrha var. *pacifica;* GOETTE, 1886, Sitz. d. könig. preuss. Akad. d. Wiss. z. Berlin, Bd. 89, p. 4.

Dactylometra pacifica var. *ferruginaster;* MAAS, 1909, Abhandl. d. math.-plys. Klass. d. K. Bayer. Akad. d. Wissen., Suppl.-Bd. 1, Abhandl. 1, p. 44, Taf. 2, Fig. 14.

Dactylometra longicirrha; KISHINOUYE, 1892, Dobutsugaku Zassi, vol. 4, p. 261, pl. 2.
Dactylometra ferruginaster; KISHINOUYE, 1892, Dobutsugaku Zassi, vol. 4, p. 263, pl. 3.

According to Mr. S. KOKUBO, the medusa is commonly found at Asamushi from spring to winter. I have examined numerous specimens from various localities of the country, such as Aomori, Misaki, Kanazawa, Seto, the Inland Sea, Kagoshima, and Toyama and have come to the conclusion to reduce several synonyms given above into one, viz. *Dactylometra pacifica,* concurring with BIGELOW (1913). However, *Kuragea depressa,* which was referred to the above species by him seems to me to be a quite different species. The original describer of the species was GOETTE, who gave it a brief description in 1886, based on a preserved specimen obtained at Nagasaki, but KISHINOUYE (1892), who examined specimens from the Inland Sea, described the species in detail.

19) **Cyanea capillata** ESCHSCHOLTZ.

Cyanea capillata: ESCHSCHOLTZ, 1829, Syst. der Acalephen, p. 68.
Cyanea capillata (in part); MAYER, 1910, Medusae of the World, vol. 3, p. 596.
Cyanea capillata var. *capillata:* BIGELOW, 1913, Proc. U. S. Nat. Mus., vol. 44, p. 93, pl. 4, fig. 8, 9.
Cyanea ferruginea; ESCHSCHOLTZ, 1899, System der Acalephen, p. 70, Taf. 5, Fig. 1.
Cyanea citrea; KISHINOUYE, 1910, Jour Coll. Sci, Imp. Univ. Tokyo, vol. 27, art. 9, p. 16, pl. 4, fig. 16, 17.

The umbrella is quite flat and discoidal, high in the central and the marginal (especially the adradial) parts, making a shallow groove on the exumbrella. It measures 350 mm. in diameter. The umbrella margin is divided into 16 lobes by deeper adradial clefts and shorter perradial and interradial sensory ones. The adradial clefts measure 45 mm., while the sensory ones 30 mm. These lobes are again divided into two, the one nearer to the sensory organ being smaller than the other.

The exumbrella is generally smooth, but the subumbrella is very complicated, as follows. The eight sensory organs are found deep at the bottom of a groove formed by two ephyral lobes and an exumbrella roof. The gastrovascular system in a lobe is characteristic in the species; it consists of a broad canal adjacent to the sense organ and about ten radial canals in a group. The broad canal sends out several canals on both sides, among them, those nearer to the sensory

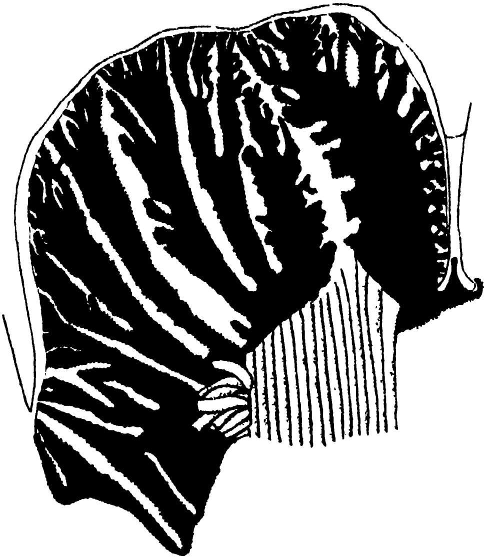


Fig. 5. *Cyanea capillata* Canal system of an ephyral lappet.

organs are larger and more numerous than those on the other side. The other radial canals are mostly dendritic, especially in the terminal portion, and sometimes are anastomosed. The peripheral margin of these canals are also provided with numerous minute canals. There are sixteen bundles of radial muscular ridges in the subumbrella between the per- (or inter-) radial and the adradius. A bundle is 60 mm. long and 30 mm. wide, consisting of 15 muscular ridges. These muscular ridges are provided with several simple transverse canals. Outside of the stomach there are parallel, concentric muscular ridges, among them, 15 peripheral are conspicuous and 5 proximal are faint. The zone of the muscular ridges is 40 mm. wide and is divided into 16 groups; eight in each per- (or inter-) radius and another eight in each adradius. Those in the perradius and the interradius are longer, 60 mm. in length, while those in the adradius are shorter, 30 mm. These concentric muscle ridges also have several transverse canals which are wider than those in the radial ridges and are somewhat arboraceous in their terminal portion. There are numerous tentacles which are arranged in about five rows along these radial and concentric muscle zones in each adradius. The tentacles are hollow and very contractile. The mouth is four-sided and is provided with four perradial oral arms, which are curtain-like and complexly folded, especially at the free margin. The stomach is 130 mm. in diameter and gives rise to 16 radial pouches, which again send out several radial canals into the lobes. The genital porticuses are somewhat isosceles triangular, though the periphery is round. The gonads are linear, imbedded in the gelatinous substance, which generally swells out from the four interradial genital porticuces, like a hollow bag.

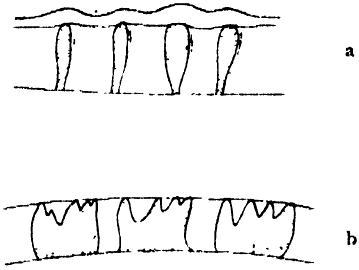


Fig. 6. *Cyanea capillata.* a. Canals of a radial muscular ridge; b. Canals of a concentric muscular ridge.

Colour. Tentacle brick brown. Muscular system and gonad yellow in preserved specimens.

Loc. Tsubakiyama, May 11, 1926.

Remarks. Medusae belonging to the genus were described as several different species from several localities. Mayer (1910) reduced these species into two: *Cyanea capillata* and *Cyanea annaskala.* Bigelow (1913) followed him and described the Japanese species in the Inland Sea as *Cyanea capillata* var. *nozakii.* Stiasny (1921) tried another revision of the genus but used the name for the medusa. However, I am of the opinion that two medusa, namely *C. capillata* and *C. nozakii,* must be separated, because they are distinguishable in the radial canals, the radial muscle system, and the colour.

The identification of species belonging to the genus has formerly been made based on their colour, or size, and the shape of the marginal lobes, but these factors are highly variable in one species, so special observations must be made on the canal systems and the muscular system, as they are stable in a preservative. With regard to the radial canals of *Cyanea capillata,* there are some differences in former figures, but those made by Kishinouye (1910) and Bigelow (1913) almost agree with the canals in my specimens.

ORD. RHIZOSTOMAE.

20) **Rhopilema esculenta** var. **asamushi,** n. var.

The umbrella is higher than a hemisphere, 100–200 mm. in diameter. The gelatinous substance is rigid, and thick at the center, becoming gradually thin towards the margin. The margin of the umbrella is divided into 64 or more velar lappets, which are often cut again. They are highly variable, not only in individuals but even in octants in a specimen. These velar lappets are oval, while the eight ocular lappets are smaller in size and more or less lanceolate. The thin marginal portion, which is turned inside and is velum-like, is 32 mm. wide in a medusa with a diameter of 180 mm. The subgenital porticus in each interradius is large and somewhat kidney-shaped. There is a wart-like protuberance, a papilla, in each interradial margin of the porticus. Its outer surface is concave inwards. The oral pillar in each perradius is almost an isosceles-triangle in cross section, having one outer surface and two inner ones. The upper portion of the oral

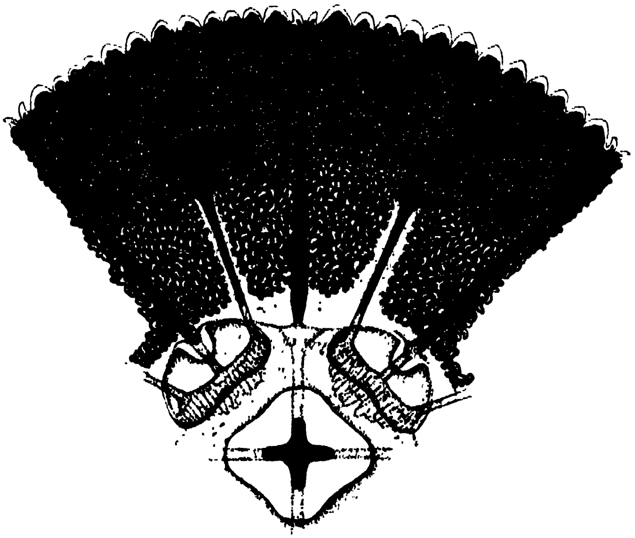


Fig. 7. *Rhopilema esculenta* var. *asamushi,* n. var. Ventral view of a quadrant of the umbrella. × $\frac{2}{3}$

part is 100 mm. in diameter and is connected with the quadrate prismatic part downwards. The prismatic part is 40 mm. in diameter and has a canal in the central portion. The canal is cruciform in section and sends off 8 pairs of canals, one into each scapulet, which is furnished with complicated sucking frills and numerous hollow filamentous appendages. Each scapulet is divided into two pairs at the distal end. The cruciform canal above mentioned is divided into 12 canals, three in each perradius. Out of these canals, a middle one leads downwards, while two paired ones run in a scapulet respectively, being divided again into two canals at its distal end. The un-paired middle canal is divided again in a lower portion into three canals: one middle and two paired. The former leads to the underside of the oral part, while the latter ones run into the oral arm, which hangs below. The ventral side of the oral arm is very similar to *Rhopilema esculenta*. There are five separate groups of sucking frills,—one smaller one in the center and four larger ones in each perradius, each provided with a long filamentar appendage. The central frill is connected by four narrow arms with the four perradial ones, which are again connected by two arms with those of the eight arms. Among the five filamentar appendages, the central one is the largest and longest.

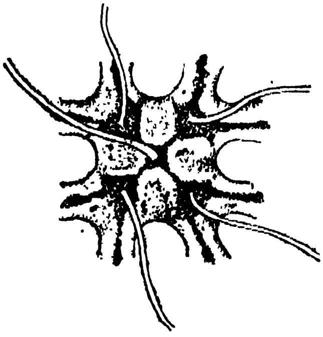


Fig. 8. *Rhopilema esculenta* var. *asamushi*, n. var. Ventral view of oral part.

The eight oral arms are not so well developed as in *Mastigias*. They are three-winged and are furnished with numerous hollow appendages which are distributed among the sucking frills of each wing. They are smaller and filamentar in the upper region but are stout and spindle-shaped. The larger ones are generally present near the terminal portion of the oral arm, but none could be specially called the terminal appendage, as in *Mastigias* and *Rhizostoma*. The oral arm and the largest appendage are almost similar in length, measuring 90 mm. The central canal of the arm gives off a few broad canals on either side, which extend to the wings and are divided into numerous minute canals which lead to a suctorial mouth.

The gastrovascular system of the medusa is characteristic. There are eight perradial and interradial canals which extend from the central stomach to the eight sense organs. Besides these eight canals, eight narrower and shorter adradial canals come off from the stomach and connect at a swollen end with the indistinct ring canal. Inside the ring canal, there are anastomosed canals, which are connected with the eight perradial and interradial canals but not with the eight adradial canals. Outside the ring canal, there are numerous narrow canals, which are more complexly anastomosed than those inside the canal.

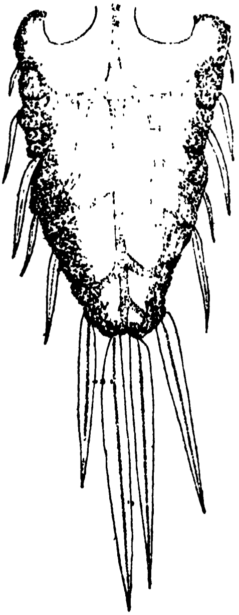


Fig. 9 *Rhopilema esculenta* var. *asamushi*, n. var. Frontal view of oral arm. $\times \frac{2}{3}$

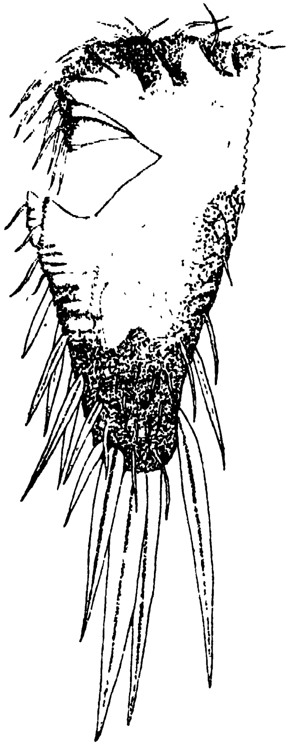


Fig. 10. *Rhopilema esculenta* var. *asamushi*, n. var. Lateral view of oral arm. $\times \frac{2}{3}$

The central stomach cavity is almost octagonal in outline and is provided with numerous gastral filaments in each interradius.

The gonads develop in each interradius, showing numerous foldings, which are larger towards the middle portion.

Colour. The umbrella and the oral arms are sandy colour and the gonads are yellowish brown.

Loc. Asamushi, Aug. 29, 1925.

Remarks. It is rather seldom that the medusa is found in the bay, but I saw numerous medusae belonging to the species at Yunohama, Yamagata Prov. The medusa is very similar to *Rhopilema esculenta*, which is common in the Inland Sea and on the coasts of Kiushu, but differs in colour and in the shape of the larger appendages of oral arms. In respect to the latter character, the medusa is somewhat common to *Rh. verrilii*, which was recorded from the Atlantic, but is easily distinguished from the latter by the presence of filamentar appendages on the oral arms and the ventral side of the oral part, and the concentric muscle, which is not interrupted in the radii of the 16 radial canals.

Ctenophorae.

21) Hormiphora palmata CHUN.

Hormiphora palmata; CHUN, 1898, Die Ctenophoren der Plankton-Expedition, p. 17, Pl. 3, Fig. 1, 2. KOMAI, 1918, Ann. Zool. Jap., vol. 9, p. 451, pl. 7, fig. 1.

Hormiphora japonica; MOSER, 1908, Abhandl. K. Bayer. Akad Wiss. Suppl.-Bd. 1, Abhandl. 4, p. 10 pl. 1, fig. 6-8.

A specimen of the common species was obtained by Mr. TAKATSUKI. The medusa is 46 mm. long and 20 mm. wide. Comb plates, 75 in number. Tentacles torn off.

Colour. Tentacle bases yellowish. Comb plates white.

Remarks. The species was recorded from the Pacific coasts of Japan by MOSER (1908) and KOMAI (1918).

I examined several specimens obtained at Toyama Bay, the Japan Sea.

Loc. The quay of the Asamushi Marine Biological Station, May, 1926.

22) **Beroë ramosa** KOMAI.

Beroë ramosa; KOMAI, 1921, Ann. Zool. Jap., vol. 10, p. 17, fig. 2.

Four specimens caught by Mr. TAKATSUKI were examined by me. The largest specimen among them is 55 mm. long and 25 mm. wide. The gelatinous substance is thick at the upper portion and becomes gradually thin towards the bell margin. Comb plates so numerous and closely set that one can hardly count them. Meridional canals, as well as pharyngial canals, giving off numerous side-branches on either side; these branches generally run horizontally into various depths of the gelatinous substance, repeatedly branching.

Colour. Slightly pinkish.

Loc. The quay of Asamushi Marine Biological Station, Sept. 5, 1926.

Remarks. The species was described in detail by KOMAI (1921) based on several specimens from Hokkaido.

LITERATURE CITED.

1) BIGELOW, H. B. 1909. Reports on the scientific results of the Eastern Tropical Pacific, 1904-1905. The Medusae. Mem. Mus. Comp. Zool. Harvard Coll., vol. 37, p. 1-243, pl. 1-48.

2) BIGELOW, H. B. 1913. Medusae and Siphonophorae collected by the U. S. Fisheries steamer *Albatross* in the Northern Pacific, 1906. Proc. U. S. Nat. Mus., vol. 44, p. 1-119, pl. 1-6.

3) FEWKES, J. W. 1887. A new Rhizostomatous Medusa from New England. American Jour. Sci., vol. 33, p. 119-125, pl. 5.

4) GOETTE, A. 1886. Verzeichniss der Medusen, welche von Dr. Sander, Stabsarzt auf S. M. S. "Prinz Adalbert" gesammelt wurden. Sitz. d. könig. preuss. Akad. d. Wissen. z. Berlin, Bd. 39, p. 1-7.

5) KISHINOUYE, K. 1892. Zwei neue Dactylometren (in Japanese, with German diagnosis). Dobutsugaku Zassi, vol. 4, p. 1-5.

6) KISHINOUYE, K. 1899. Edible Medusae. Zool. Jahrb., Abt. f. Syst., Geog. u. Biol. d. Tier., Bd. 12, p. 205-209, Taf. 13.

7) KISHINOUYE, K. 1910. Some Medusae of Japanese Water. Jour. Coll. Sci., Imp. Univ. Tokyo, vol. 27, Art. 9, p. 1-33, pl. 1-5.

8) KOMAI, T. 1918. On Ctenophores of the Neighbourhood of Misaki. Ann. Zool. Jap., vol. 9, p. 451-473, pl. 7.

9) KOMAI, T. 1921. Notes on two Japanese Ctenophores, *Lampetica pancerina* Chun and *Beroë ramosa* n. sp. Ann. Zool. Jap., vol. 10, p. 15-18.

10) MAAS, O. 1909. Japanische Medusen. Abh. K. Bayer. Akad. Wiss., Suppl.-Bd. 1, Abhandl. 8, p. 1-52, pl. 1-3.

11) MAYER, A. G. 1910. Medusae of the World. vol. 1-3, p. 1-735, pl. 1-76.

12) MOSER, F. 1908. Japanische Ctenophoren. Abh. math. phys. K. Bayer. Akad. Wiss., Suppl.-Bd. 1. Abh. 4.

13) OKADA, Y. K. 1926. Le genre Spirocodon (hydroméduse). Description d'une nouvelle espèce. Ann. Zool. Jap., vol. 11, p. 75-85, pl. 1.

14) OKUBO, T. 1917. Preliminary Note on a New Genus of Stauromedusae from Hokkaido (in Japanese, with English diagnosis) Dobutsugaku Zassi, vol. 29 p. 317-322.

15) STIASNY, G. 1920. Results of Dr. E. Mjöbergs Swedish Scientific Expeditions to Australia 1910-13. Pt. 30, Scyphomedusen. Kungl. Svenska Velenskapsakadem. Handlingar. Bd. 62, p. 1-13.

16) UCHIDA, T. 1925. Some Hydromedusae from Northern Japan. Jap. Jour. Zool., vol. 1, p. 77-100.

17) UCHIDA, T. 1926. Notes on a Young Specimen of Cubomedusae. Proc. Imp. Acad. Japan, vol. 2, p. 296-298.

18) UCHIDA, T. Japanese Anthomedusae with Remarks on their Natural Relationships. (Im press).

The Anatomy of *Caudina chilensis* (J. MÜLLER) with Especial Reference to the Perivisceral Cavity, the Blood and the Water Vascular Systems in their Relation to the Blood Circulation.*

BY

NOBUYUKI KAWAMOTO.

This investigation was undertaken at the suggestion of Prof. S. HATAI as the first step for further investigation on the physiology of the blood, during my stay at the Asamushi Marine Biological Station from the middle of March to the end of October 1925.

MATERIAL AND METHOD.

The systematic position of *Caudina chilensis* (J. MÜLLER) is as follows:

Phylum. Echinodermata.
  Class. Holothurioidea.
    Order. Molpadiidæ.
      Genus. *Caudina.*

This animal lives in the littoral sandy bottom of the sea, and is found in abundance in the Bay of Moura, near the Asamushi Marine Biological Station. This species was collected also at Hokkaido, according to MITSUKURI (1).

As this animal contracts strongly when dug out of the sand, I put it into a bath through which sea water ran slowly for a while for the purpose of recovering its natural form. In order to narcotize it, a small amount of magnesium sulphate was gradually added the bath in which the *Caudina*, thus recovered, was kept. After the *Caudina* was sufficiently narcotized, it was suddenly removed to saturated picric acid solution for the purpose of fixation. The fixation of its alimentary canal was very difficult as it contracts irregularly, but I succeeded by cutting off a portion of the canal, cleaning away the sand which filled it, then binding one end with string and, finally, the other end was bound after it was filled with picric acid solution with a pipette. The intestine thus prepared was finally fixed in *toto* in the same picric acid solution.

---

* A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

The materials were imbedded in paraffin and were sectioned at the thickness of 3 to 20μ for the purposes of the investigation.

Eosin and Delafield's hæmatoxylin were found the most suitable stains for the purpose of examining the blood vessel.

For the study of the blood corpuscles in both fresh and fixed conditons I used several kinds of stains. Of course the fixing methods of the blood corpuscles were different according to the different stains. Methyl alcohol was used for the Giemsa's stain, formalin for the May-Greenwald's stain, osmic acid of the Weidenreich method for the methylgreen-pyronin of Pappenheim-Unna, for the alcoholic eosin solution and for Delafield's hæmatoxylin.

Thoma-Zeiss's hæmocytometer was used for the enumeration of the corpuscles.

ANATOMY.

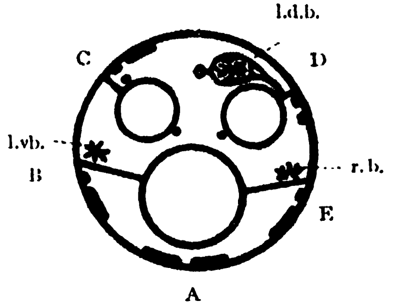
External features.

The general appearance of the body is spindle shaped, tapering rapidly at the anterior end and much elongated and slender at the posterior end, which is called the tail. (Plate 1, Tl.) The mouth (Plate 1, M.) opens at the anterior end, and is surrounded by fifteen oral tentacles (Plate 1, T) of equal size. The anus (Plate 1, A) opens at the extreme end of the tail. Four cone-shaped lobes are found at the free end of each oral tentacle.

The number of tentacles of this species is fifteen, the same number as GEROULD found in *Caudina arenata.* A short cone-shaped genital papilla (Plate 1, G. p.) is situated near the mouth along the dorsal interradius, and it appears to be slightly longer and more slender in the male than in the female. At the extreme end of the tail particles of sand are attached which give a much darker color. I suppose this may be for protection from enemies. On the extremity of the tail, along each radius, there is found an anal papilla. There are five in the adult, but they are hardly visible to the naked eye. (Plate 2, Fig. 5, a.P.)

The body wall is semi-transparent and on the inner surface of it five pairs of longitudinal muscles are attached in positions which correspond to the ambulacral zones.

The tube feet are absolutely wanting. Each pair of longitudinal muscles show a difference in width from the others at the middle of the body (Text Fig. 1, A, B, C, D, E) although this difference becomes gradually indistinguishable as these approach toward both ends. The width of the ventral longitudinal muscles, which are located at the middle of the ventral inner surface, is the same. Of the muscles which are located at both sides of the longitudinal muscles, the ones near the ventral median line are wider than the others, and of the left dorsal longitudinal muscles, which are located left of the dorsal median line, the one near the line is wider than the other, while the corresponding pair of muscles, which located at the right side of the dorsal median line is nearly identical in width. Thus we can easily distinguish dorsal or ventral, left or right of the body by examining the five pairs of longitudinal muscles, as these differences can be seen from the surface of the body. In order to designate each pair of longitudinal muscles, I have called the ventral longitudinal muscles A, the left ventrals B, the left dorsal C, the right dorsal D, and the right ventral E. (Plate 1, L. m.)



Text Fig. 1. Diagrammatic section of the body.

Internal features.

1. Alimentary canal.

This canal, after passing through the calcareous ring which is located near the mouth, runs (Plate 1, c. R) towards the posterior end of the trunk, then bends and runs forwards almost to the calcareous ring, then turns again towards the tail and finally opens at the anus. Thus the general form of the alimentary canal in its natural position resembles the letter S.

The first three-fourths of the canal is attached to the body wall by means of membranes, termed mesenteries (Plate 1, Me.). There are four such mesenteries, the first one supports the anterior one-fourth of the canal left of the D muscles. The second one supports the next one-fourth of it to the left of the C muscles, and the last two mesenteries support the next one-fourth of the canal on the upper side of the B and the E muscles (Text Fig. 1). The remaining one-fourth of the canal runs through the tail and is supported by the radial cloacal muscles. (Plate 1, R. c. m.)

The pharynx (Plate 1, Pa.) is attached to the calcareous ring by ten septa. These ten septa are called the "suspensor" by GEROULD, but I wish to call the "cœlo-peripharyngeal septa" (Plate 2, Figs. 1 and 2, c. p. p. S.) for the reason of signifing their anatomical relations.

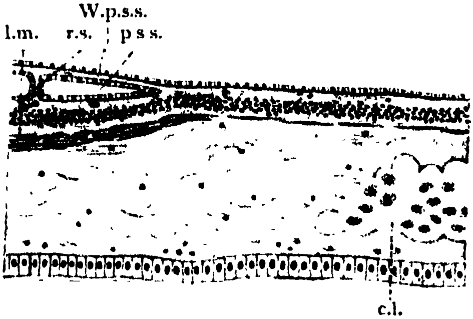
There are five peripharyngeal sinuses surrounding the pharynx and each is surrounded by a continuation of the cœlomic cavity (Plate 2, Fig. 1, p. p. S.), thus each sinus is formed by two cœlo-peripharyngeal septa, a part of the pharyngeal wall, and one piece of the calcareous ring corresponding to the radius. The posterior ends of the septa are connected with the wall of the peristomachal sinus, which is further explained later, while the anterior ends are free. At these free anterior ends the cœlom communicates with the peripharyngeal sinus and will be called the cœlo-peripharyngeal connection. (Plate 2, Fig. 2, c. p. C.)

The pharynx becomes continuous with the stomach at the posterior end of the calcareous ring (Plates 1 and 2, Fig. 2, c. R.) to which the circular water canal (Plate 1, w.) attaches. The inner surface of the pharynx has numerous longitudinal folds.

The stomach (Plate 1, St.) is a short, curved tube of uniform diameter and of whitish color. A membrane which surrounds the stomach is called the wall of peristomachal sinus and is composed of two epitherial layers and a connective layer which lies between them. (Text Fig. 2. W. p. s. s.) Between this membrane and the stomach, there is a sinus, which is here named the peristomachal sinus. (Text Fig. 2, p. s. s.) The walls of the peristomachal sinus and the stomach are connected by fibrous suspensors, which are arranged radially, though irregularly. (Text Fig. 2, r. s.)

This sinus just mentioned communicates with the peripharyngeal sinuses in the anterior, but posteriorly, near the intestine, this sinus ends in a blind sack, owing to the union of both walls. (Text Fig. 2)

The genital organ (Plate 1, g. o.) of this animal consists of two dichotomously branched tubules of almost uniform caliber. These two branches of tubules open into a common duct at the point where these tubules unite. The common genital duct runs anteriorly between the two epithelia of the mesentery, and opens to the exterior through a single orifice of the genital papilla. (Plate 1, g. p.)



Text Fig. 2. Diagrammatic section showing the connection of the stomach and the small intestine.

The small intestine has the form of a U, and is supported by the mesenteries which are attached to the C and the D muscles. As there are many capillary lacunae filled with red blood in the inner connective tissue layer of the small intestine, it can be distinguished from other parts of the alimentary canal by its color. The part which is supported by the D mesentery is called the anterior small intestine (Plate 1, A. s. i.) and the remaining part is called the posterior small intestine. (Plate 1, P. s. i.)

The large intestine (Plate 1, L. i.) runs ventral to the small intestine and ends at the anal opening. The anterior end of the large intestine down to the end of the trunk is comparativly larger in diameter than any other part of the canal, and is supported by two mesenteries which are attached to the B and the E muscles, respectivly, on both sides. Beyond this region, the large intestine becomes narrower and runs into the tail, where it is supported by the radial cloacal muscles. (Plate 1, R. c. m.)

The two respiratory trees, (Plate 1, r. T.) which run parallel, are attached to the dorsal side of the large intestine at a point near the bend of the small intestine. The left tree divides itself near its origin into two main branches, one of which goes forward along the dorsal surface of the B mesentery and may be called the left ventral branch (Text Fig. 1, l. v. b.), while the other, the left dorsal branch, (Text

Fig. 1, l. d. b.) enters the rete-mirabile and is loosely invested. (Plate 2, Fig. 3, R. M. & r. t.)

The right one, which is a single trunk, goes forward along the dorsal surface of the E mesentery and runs near the circular water canal, where it divides into two branches, the one reaches the base of the tentacular ampulla (Plate 1, t. A.) on the dorsal median line, and the other runs to the ventral median line.

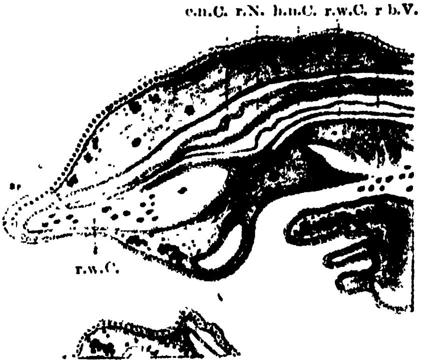
2. *Terminal portion of the tail.*

The five anal papillae (Plate 2, Fig. 5, a. P.), as I have mentioned already, are found at the posterior end of the tail as in *Caudina arenata*.

I found that the cœlomic cavity opens at the inner base of the papillæ to the exterior through five pores which I called the "cœlo-anal canals" (Plate 2, Fig. 5, & 6, c. a. C.). The presence of these canals, the diameter of which are 40–50μ in the adult, can scarcely be seen by the naked eye but occassionally their openings may be clearly seen at the instant when the red colored cœlomic fluid is thrown out. These canals are individually covered with a single layer of epithelium and are free from cuticle. I could not find any trace of muscular structure which may serve to close the pores. These cœlo-anal canals have been noted neither by GEROULD (2) in *Caudina arenata* nor by DENDY (3) in *C. coriacea*, and as far as I am aware, these canals were not found in any other Holothuroidea by other investigators.

Each of the five radial water canals (Plate 2, Fig. 4 and 6, r. w. C.) comes along the inner side of the radial nerves and divides into three branches near the base of the anal papillæ. Only the median branch enters the papilla, where it is enlarged slightly, while the other two end at the side without showing any enlargement. The radial blood vessels (Plate 2, Fig. 6, r. b. V.) run along the dorsal side of the median branch of the water canals, which is located in the papillæ. GEROULD (2) reported that in *Caudina arenata* these radial lacunæ (radial blood vessels) communicate at the top of the tail with circular lacuna, which surrounds the anal opening. This, however, is denied by DENDY (3) who could not see such lacunæ in *C. coriacea*. I also, failed to see such communications in *C. chilensis*.

Each radial nerve (Plate 2, Figs. 4 and 6, r. N.) accompanies the epineural canal (Plate 2, Figs. 4 and 6, e. n. C.) as well as the hyponeural canal. (Plate 2, Figs. 4 and 6, h. n. C.) The hyponeural canal was called by HAMMAN the pseudo-hæmal canal. The epineural canal is continuous with the circular epineural canal surrounding the mouth, which lies anterior to the nerve ring, while in the posterior portion it divides into three canals (Text Fig. 3, e. N. C.) along with the three branches of the radial water canal.



Text Fig. 3. Diagrammatic section illustrating the structure of the end of the tail.

These three epineural canals unite with the corresponding branch of the radial water canal (Text Fig. 3, c.) and forms three canals at the extremity, while the hyponeural canal communicates at certain intervals with the epineural canal in the ambulacural systems.*

BLOOD VASCULAR SYSTEM.

Distribution.

The general arrangement of the blood vascular system of this species is the same as that of the other species of Holothurioidea. Dorsal and ventral vessels lie along the intestine, and communicate with each other by intestinal lacunæ, while the posterior parts of the vessels are connected to a circular blood vessel (Plate 1, c. b. V.) which forms a ring at a region near the circular water canal, from which the five radial blood vessels go out to the ambulacural zones.

All the vessels which are found on the dorsal side of the alimentary canal are called the dorsal blood vessels (mesenterial blood vessels) and run along the dorsal surface of the alimentary canal. Numerous fine capillaries are sent out from the dorsal median line of the anterior small intestine (the left side of the mesentery) and are gradually united with one other in a network. This network, or plexus of the capillaries, is called a rete-mirabile (Plate 1, R.) and become united with a vessel which comes from the anterior portion of the alimentary canal. This vessel, after uniting with the capillaries, divides again into two vessels, one of which goes to the anterior, and the other to the posterior part of the body. The anterior one runs along the dorsal surface of the posterior portion of small intestine, branches off into many short vessels running to the dorsal median line of the intestine where the right side of the mesentery is attached. These short vessels become continuous with the intestinal lacunæ, which are situated in the wall of the posterior small intestine. The posterior one divides itself into two or three branches, which run near the bend of intestine, where they enter the intestinal lacunæ.

---

* *Ambulacural system* is a term given to the complex arrangement of the epineural canal, radial nerve, the hyponeural canal, the radial blood vessel, and the radial water canal, which is found in the ambulacural zone.

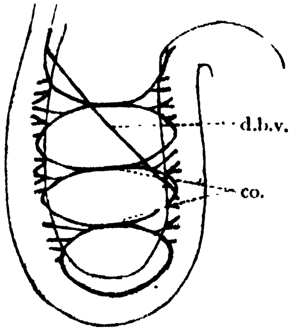
The vessel which comes from the rete-mirabile is called the afferent vessel (Plate 1, a. V.), and the two main branches are called the anterior and the posterior efferent vessels (Plate 1, a. e. V. and p. e. V.).

To these three vessels, which present a Y arrangement, I have given the name "contractile vessels" as these vessels perform rhythmical pulsation similar to the heart movement in higher animals. The anterior part of the afferent vessel becomes much more slender, runs forward, passing between the genital duct and the wall of peristomachal sinus, and then become continuous with the circular blood vessel.

The capillaries on the ventral surface of the rete-mirabile are roughly mingled with the left dorsal branch of the respiratory tree as I have already described (Plate 2, Fig. 3, R. M.).

All the vessels which are found on the ventral side of the intestine are termed the ventral blood vessels (anti-mesenterial b. v.), and to these belong the commissures (Text Fig. 4, and Plate 1, co.) and the diagonal blood vessel (Text Fig. 4, and Plate 1, d. b. v.).

The commissures join the vessels which run along both the anterior and posterior part of the small intestine. Each of these commissures collects several vessels which come from the ventral median line of the anterior small intestine, taking one vessel, and then divides gradually into several vessels, the number of which are nearly same as before the union, and enters the lacunæ of the posterior part of the intestine at its ventral median line. In the last commissures the right branches are united to the lacunæ of the anterior portion of the intestine, while the branches of the left side are united to the lacunæ of the posterior portion of the intestine. Among these branches, the anterior one goes forward along the ventral side of the stomach and reaches the circular blood vessel, while the anterior branch to the posterior small intestine reaches the beginning of the large intestine and communicates with the capillary network found in its wall.



Text Fig. 4. Diagram to show the ventral blood vessel.

Though the number of the commissures is three in normal adult forms, yet we frequently find it varies from three to seven. In the cases where the commissures are many, they are often anastomosed, and under such circumstance the exact number can not be determined. The diameter of these commissural vessels becomes smaller as the number increases, and, in general, the diameter of the central part of the commissure is larger, and the one which lies near the bend of the small intestine gives the largest diameter. The one which lies nearest the bend, or which gives the largest diameter, is called the first commissure, and the others are called the second, the third, and so on, according to the order of their position. The diameter of the central part of the first one is 0.2 to 0.9 mm. according to the individual.

The diagonal blood vessel is a vessel diagonally connecting the ventral surface of the posterior small intestine from near its bend to the beginning of the anterior small intestine. The origin of the diagonal vessel at the right side is always constant, being attached at the beginning of the anterior small intestine, but the attachment of the other end frequently varies, sometimes it is near the bend of the intestine, and sometimes at a branch of the first commissure, though, in the majority of cases, it comes between the first and the second commissures.

Gerould (2) describes that "at the bend between the small and large intestine there is a delicate sheet of anastomosing vessels," but I am not able to find such a plexus of capillaries in *C. chilensis.* GEROULD did not mention the existence of the diagonal blood vessel either in his description or in the plate, so I suppose it is lacking in *C. arenata.* It is worth noticing that this diagonal vessel, which is so characteristic and prominent in our species, is not found in the American species, *C. arenata.*

Circular and radial blood vessels.

The circular blood vessel (Plate 1, c. b. V.) is ring shaped and is situated around the pharynx, and at the posterior margin of the circular ring of the water vascular system. The cross section of this circular blood vessel is spindle-shaped, and the outer wall of this vessel is continuous with the circular ring of water vascular system. The dorsal and the ventral blood vessels, which come along the wall of the peristomachal sinus, become continuous with the circular blood vessel at the region of the dorsal inter-radius and of the ventral radius respectively. This circular blood vessel gives off the five radial blood vessels (Plate 2, Figs 4, 5, and 6, r. b. V.) which go forward along the inner surface of the radial water canal, and each of these gives three short, fine branches in company with the water canals of the tentacles. The radial blood vessels run along with the radial nerves (Plate 2, Fig. 4, r. N.) which come from the nerve ring at the anterior margin of the calcareous ring. All the structures just mentioned, together with the radial water canals, constitute the ambulacural system. The radial blood vessels run backward, and then open into the median branches of the radial water canals in the anal papillæ. (Text Fig. 3.)

The so-called muscular tubules or the muscle cylinders, which are present in the connective tissue layer of the body wall and are placed vertically to the surface, have been considered the blood vessels by some former investigators, but as far as my observations go, these are not blood vessels but are muscles, as well fixed preparations show clearly the histological characters of muscle structure.

Intestinal capillary lacunæ.

There are intestinal capillary lacunæ which communicate between the dorsal and the ventral vessels. (Text Fig. 2, c. l.) These lacunæ lie in the inner layer of the connective tissue of the small intestine, and give a red color to the small intestine due to the filling of the lacunæ with red corpuscles.

Capillary network.

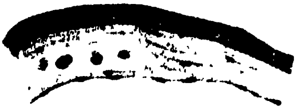
The dorsal blood vessel ends at the connection of the small and the large intestines, while the ventral vessels, an anterior branch of the last commissure, enters the large intestine a little distance beyond the ventral surface of the small intestine and communicates with the capillary net-work, which is situated in the inner layer of the connective tissue. This capillary net-work is roughly anastomosing and extends as far posterior as the tail end, and soon, after enveloping the tip of the cœlomic cavity, opens in the epineural canal by many lacunæ that lie between the circular muscle and the connective tissue of the tail wall.

The epineural canal communicates with the radial water canals in the anal papillæ, as described before, and, since the radial water canal opens into the perivisceral cavity through the circular water canal, which, in turn, opens to the stone canal (Plate 1, S.) and the madreporite. (Plate 1, Md.) The capillary network and the radial blood vessels ultimately connect with the perivisceral cavity by means of the radial water canal. The wall of the large intestine is not red in color owing to the presence of a smaller amount of blood in the capillary network. I met only one instance of the red colored blood vessel which goes backward along the ventral surface of the large intestine to the attachment of the water lungs, as was illustrated by GEROULD in his work in the plate, while normally it ends abruptly at the beginning of the large intestine.

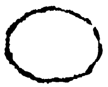
On the structure of the blood vessels and the intestinal lacunæ.

The four kinds of blood vessels and lacunæ may be distinguished from each other according to the histological point of view: 1) The wall of the vessels, which consists of six kinds of tissue layers. 2) That which consists of two epithelial layers, 3) That which consists of a single layer of epithelium, 4) That which consists of lacunæ.

1) The majority of the dorsal and the ventral blood vessels belong to this type of vessel (Text Fig. 5), and consist of an outer epithelial layer composed of one layer of cells (in the diagonal vessel the cells are cylindrical, though in all the other cases the cells are flat), a thin outer layer of connective tissue, a very thin layer of longitudinal muscle fibers, a layer of circular muscle fibers, an inner layer of connective tissue, which is the thickest layer of all and in which often the lacunae containing red corpuscles are found, and a thin epithelial layer called the *intima.*

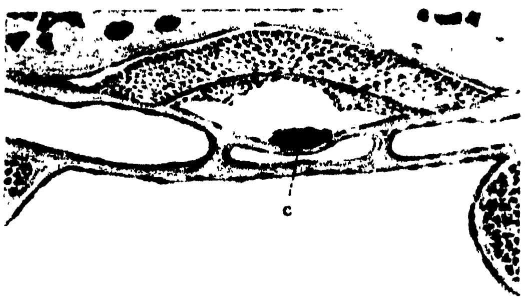


Text Fig. 5. Diagrammatic section to show the wall of the contractile blood vessel.

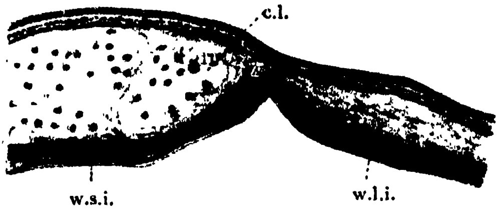


Text Fig. 6. Diagram to show the cross section of a capillary of the rete-mirabile

2) The rete-mirabile belongs to this type and is composed of two epithelial layers. I could not find any other tissue between these two layers (Text Fig. 6). This type gradually transforms into the former type as the rete-mirabile aproaches the contractile vessel. 3) The very thin capillaries, a circular blood vessel, a radial blood vessel belong to this type (Text Fig. 7, c.). 4) The lacunæ lie in the connective tissue layer. Their walls are identical with those of the capillaries or their anastomoses, therefore, I called them capillary lacunæ. (Text Fig. 8, c. l.)



Text Fig. 7. Cross section of the ambulacural system to illustrate the radial blood vessel. (c.)



Text Fig. 8. Cross section of the connection of the large and the small intestines to illustrate the capillary lacunæ. (c. l.)

The part of the dorsal and the ventral blood vessels which form the part of the wall of the peristomachal sinus, and the intestinal capillary lacunæ in the inner connective tissue of the small intestine belong to this type. The intestinal capillary lacunæ are spaces of large size, though in the connective tissue layer of the large intestine, they are usually very small and anastomoses roughly.

I examined with utmost care whether or not valves exist in the blood vessels, but thus far I could not find such structures in the blood vascular system of this species.

CORPUSCLES IN THE PERIVISCERAL CAVITY, THE BLOOD AND THE WATER VASCULAR SYSTEMS.

From the morphological point of view, six kinds of corpuscles may be distinguished in the perivisceral cavity, the blood, and the water vascular systems: red, white, minute, brown, fusiform, and crystal corpuscles.

a. Red corpuscles. The red corpuscles of *Caudina* resemble those of vertebrata in color, being pale green (Plate 3, Fig. 1, a.) when seen singly, but crimson lake or maroon color when massed together. The normal red corpuscles are round and biconvex and the diameter of the corpuscles is 10–12μ and their thickness 4-6μ. The corpuscles found in the perivisceral cavity and in the water canals are usually spindle in shape and show many other irregular forms. The irregular corpuscles send off one or several processes from the poles, and on account of the presence of spindle formed corpuscles in the perivisceral cavity, are often taken by casual observers as the normal form of red corpuscles. Among the spindle-shaped corpuscles the larger specimens measure 18 × 10μ, while the smaller measure 12 × 7μ and the length of its process is often three times the length of the greatest diameter of the corpuscles. The process resembls the flagellum of unicellular organisms. However, it is more proper to consider it a mere process, because it becomes short when treated with hypotonic salt solution, owing to the expansion of the cell body by osmotic pressure.

Further more I have not directly observed the neoformation of the process outside the cell body. For these reasons I think that the process is neither a flagellum in its structure nor is it a pseudopodium, as I have not noticed any active motion, as for instance, one find in the amœba.

In the center of the corpuscle a bright yellowish brown granule, 2–4μ in diameter, (Plate 3, Fig. 1, a.) is usually found but in some instances it is either totally absent or two are found. This granule is easily mistaken for a nucleus from its similar shape. The usual form of the granule is round, and I rarely saw them much larger in size or constricted in shape. In my opinion, it is highly probable these granules are what DENDY (3) described as the "highly refractive particles" in the red corpuscle of *C. coriacea*. I sometimes observed a few little colorless granules, 1–2μ in diameter, together with ordinary granules in the red corpuscles of the blood vessels.

When we fix the corpuscles with various kinds of reagents, we see a clear spherule in the center of the corpuscle which can not be seen in the fresh material. However, its presence becomes evident when the corpuscle is either dead or in stale. This clear spherule is well stained by Delafield's hæmatoxylin, but not at all by eosin (Plate 3, Fig. 5, a.) and organge G; the methyl-green-pyronin stains it a violet color; (Plate 3, Fig. 3, a.) May-Greenwald's method stains it also a violet color but the center of the spherule remains unstained; (Plate 3, Fig. 2, a.) Giemsa's method stains the spherule a blue color. (Plate 3, Fig. 4, a.)

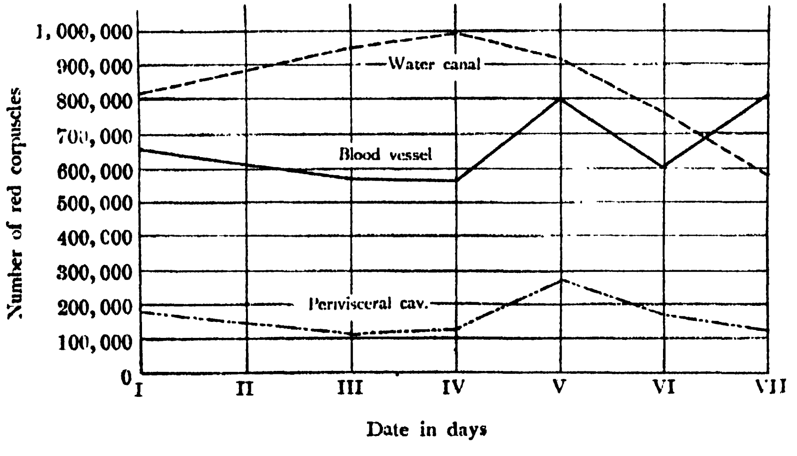
From these observations I conclude that this clear spherule is the nucleus. GEROULD found the nucleus of *Caudina arenata* to be a yellowish brown granule. I further conclude that the portion in the nucleus unstained by May-Greenwald's method corresponds to a nucleolus.

From the fact that the red corpuscles are well stained by the alcoholic solution of eosin, except a nucleus and a surface zone, (Plate 3, Fig. 5, a.) I think that the corpuscle has an exosoma and an endosoma, and that the process belongs to the former. The plasma of the corpuscle is not stained by Delafield's hæmatoxylin, but becomes reddish sepia by methyl-green-pyronin, a pretty crimson lake by May-Greenwald's, (Plate 3, Fig. 2, a.) and a pink color by Giemsa's method. The yellowish brown granules are always unstained by any method so far applied..

In the blood vessels only round and biconvex red corpuscles are found, while in the water canal spindle-shaped corpuscles are chiefly found, though the processes are totally absent or are very short, and only a few round biconvex corpuscles. In the perivisceral cavity the number of round and biconvex corpuscles extremely small and the spindle or other irregular shaped corpuscles, with long processes, are chiefly found. From the average count on 20 individuals I found that the number of red corpuscles in the blood vessels runs as high as 660,000, in the polian vesicle of the water canal 800,000 and in the perivisceral cavity 180,000 per 1 cmm.

Caudina can not live in the aquarium over 1 or 2 weeks even a favourable temperature, though the real causes of death have not yet been determined. It occurred to me that the determination of the

TEXT FIG. 9.



number of corpuscles during these period may be of some interest in the further study of this important problem. For this purpose I examined three or four individuals daily and obtained the results shown in the text fig. 9. We notice that the number of red corpuscles in the blood vessels increases during the first seven days of captivity, but we must wait for further careful investigation before we can state the meaning of this increase.

b. White corpuscles with spherules. The white corpuscles in normal condition measure 10–15μ in diameter. They contain many colorless spherules 1.5–2μ in diameter, and show movement on the slide glass by means of lobe-shaped pseudopodia. (Plate 3, Fig. 1, b.) There is still another kind of corpuscle which moves more activly and in which the spherules are more sparsely distributed than in the white corpuscles with spherules, described above, and these show much more irregular forms. These two kinds of corpuscles may have a genetical relation to each other as is indicated by the identity of the staining reactions. The white corpuscles are not stained at all by either eosin or orange G, but spherules are stained dark violet by Delafield's hæmatoxylin, violet blue by May-Greenwald's method which a few vacuole-like spherules are seen without any color, (Plate 3, Fig. 2. b.) reddish violet by methyl-green, (Plate 3, Fig. 4, b.) and also violet by Giemsa's method.

I think the white corpuscle with spherules is basophilic, with the exception of the methyl-green reaction. In the wall of the alimentary canal and in the inner layer of the connective tissue of the body wall there are many wandering cells. As these cells contain eosinophilic spherules they may be a kind different from the white corpuscles, though the forms resemble each other. But KINDRED's investigation (4) on *Arbacia sp.* suggests that even these two kinds of corpuscles just mentioned may be related genetically. The average number of white corpuscles is 70,000 per 1 cmm.

c. Brown amœboid corpuscles. The brown amœboid corpuscles exhibit an irregular form, contain many brown spherules in a colorless plasma (Plate 3, Fig. 1, d.) and possesses several spine-shaped pseudopodia. This corpuscle moves actively and is smaller than the red corpuscle. The average number of these corpuscles per cubic centimeter is found to be 6,000. These corpuscles are contained in the perivisceral cavity and in the water canal, but not in the blood vessels. The spherules of these corpuscles are stained black in color by May-Greenwald's method, brownish black by methyl-green-pyronin and a nucleus-like substance is also seen by this method, brownish black by Delafield's hematoxylin, black by Giemsa's method, and unstained by eosin. (Plate 3, Figs. 2, 3, 4, d.)

d. Crystal corpuscles. H. Théel (5) observed crystal corpuscles in the cœlomic fluid of *Cucumaria lactea, C. elongata, C. hyndmani,* and *Thyone fusus,* and he quoted in his paper that Semper (1864) has investigated the crystal corpuscles of *Thyonidium cebuense, Cucumaria canescens, Holothuria coluber,* etc. I also found crystal corpuscles in the cœlomic fluid of *Caudina chilensis,* but never in the water canal nor in the blood vessels. The crystal corpuscle is a transparent cell which contains a colorless rhomboidal crystal 8–10μ in long axis, 4–5μ in short axis, and 2–10μ in height.

Sometimes I saw the crystal was double (Plate 3, Fig. 1, e.), and it is always situated on one corner of the cell body and in the center of it is found a small bright granule. The crystal did not change at all even when it was heated in the flame of the gas burner. The plasma of the cell-body is stained red by eosin when fixed with dry heat, and violet by May Greenwald's method, but the crystal can not be stained at all.

e. Minute corpuscles. Colorless, small corpuscles, measuring 1–4μ in diameter in which any structure can not be seen (Plate 3, Fig. 1, f.) resemble minute hyaline corpuscles from the cœlomic fluid of *Thyone fusus,* which Théel described. Théel said about these corpuscles that "they are probably to be considered as protuberances or remains of them, disentangled from the blood corpuscles" and I suppose they may be the nuclei of disintegrated red corpuscles, though the reaction of the staining differs from the staining of the nuclei found in the cell-bodies. These are stained red by eosin, dark reddish violet by methyl-green-pyronin, light violet by May-Greenwald, and violet by Giemsa's method. (Plate 2, Fig. 3, f.)

f. Fusiform corpuscles. The fusiform corpuscles are colorless and exhibit no definite structure. They measure 10–30μ in length, and 3–8μ in width and are commonly found in the water canal, and in some cases, in the perivisceral cavity, but never in the blood vessels.

THÉEL said that it is "probably a fragment of shattered tissue" to which explanation I also agree. This is unstainable by eosin and Delafield's hæmatoxylin, but is stained reddish violet by Giemsa, methyl green and May-Greenwald's method. (Plate 2, Figs. 1, 2, 3, 4, c.)

Beside these corpuscles I observed masses of brown colored spherules both in the blood vessels, and the perivisceral cavity, especially in the polian vesicle. HOWELL (6) found them also in *Cucumaria sp.*, but he could not find the meaning. GEROULD (2) said in *Caudina arenata* that these masses may be an excretory product. I have no opinion about this except that the mass is much like the brown granules which are normally present in the center of the red corpuscle not only in form and in size but even in the staining reaction.

COAGULATION.

I could not see the coagulation of the cœlomic fluid or even of the blood in *C. chilensis* though THÉEL found this phenomenon with the blood of *Mesothuria intestinalis*. HOWELL (6) observed in the blood of *Cucumaria sp.* that "specimen of the perivisceral fluid allowed to stand in watch-glass an imperfect coagulum, the corpuscle setting to the bottom as a membranous like sediment, and the imperfect coagulum is formed by the fusion of the amœboid white corpuscles or of thick pseudopodia which arise from them." I experimented with the cœlomic fluid of *C. chilensis* and found that when the corpuscles are deposited for a few minutes a membrane-like sediment is formed at the bottom, but it is so feeble that it broke at once when I shook the vessel. But as I did not see that any plasmodium was formed by white corpuscles, some other coagulable substance may be present in the fluid.

The white corpuscles make a mass mixed with the red corpuscles, and I sometimes saw many such masses of various sizes in the fresh cœlomic fluid, though not very often. This mass does not seem to increase in size when the cœlomic fluid which contains it is left aside for a while. GOODRICH (7) reported that the plasmodia of invertebrata serves not only to close wounds, but also to surround foreign particles or parasites too large to be dealt with by a single leucocyte.

COURSE OF THE CIRCULATION OF THE BLOOD.

Athough the fluid in the blood vessel, in the water canal and in the perivisceral cavity shows a red color, the corpuscles contained in the fluids are different according to the place located, as is shown in Table 1.

TABLE 1.

	Blood vessel	Water canal	Cœlomic cavity
Red corpuscles	"	"	"
White corpuscles	"	"	"
Minute corpuscles	"	"	"
Brown corpuscles	—	"	"
Fusiform corpuscles	—	"	"
Crystal corpuscles	—	—	"

The brown, the fusiform, and the crystal corpuscles are not found in the blood vessels, and the crystal corpuscles are alone absent in the water canal, while all kinds of corpuscles are present in the cœlomic cavity.

From this relation, it may be supposed that the blood flow occurs from the blood versel to the cœlomic cavity through the water canal, viceversa.

In order to ascertain the direction of the blood flow, the body was opened and the vascular system of the madreporic portion was studied by means of a biocular microscope, and I could observe several times the blood flow into cœlomic cavity from water canal through the madreporite, therefore it is supposed that the blood flow occurs from water canal to the cœlomic cavity, but not to the blood vessel.

I attempted to determine more precisely the normal course of the blood circulation by observing it directly from the surface of the vessels or by injecting many kinds of dyes or other suitable stuffs into the perivisceral cavity and into the blood vessls.

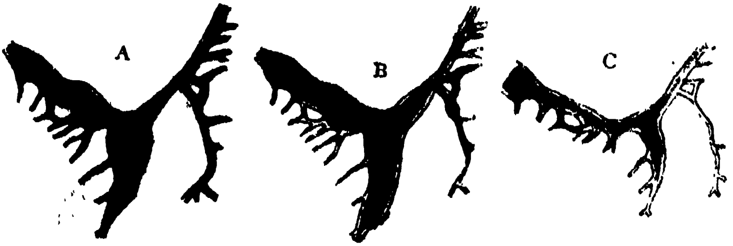
In the first place I injected the following materials into the perivisceral cavity from the body surface in order to determine whether or not the fluid of the perivisceral cavity enters either the water canal or the blood vessel.

Chinese ink dissolved in isotonic salt-solution.
Carmine solution in isotonic salt-solution.
Beale's Prussian blue.
Ranvier's Prussian blue glycerin mass.
Salt-solution in which red corpuscles stained with eosin were mixed.

I did not see any of the dye stuff at all entering the water canal or the blood vessel. I, however, found many stained corpuscles attached to the surface of the madreporite, which may be taken to indicate that the fluid entered the water canal from the perivisceral cavity. In the next place, I observed the blood flow from the surface of the blood vessels. As the rete-mirabile forces the blood forward to the afferent vessel by the pulsatory movement of the constriction of the capillaries, the afferent part of the contractile vessel expands, as is shown in Text Fig. 10 from A to B. The pulsation of the afferent vessel gradually moves on to the efferent and then into the intestinal lacunæ. (Text Fig. 10, c.)

The pulsation or expansion and contraction occurs in irregular intervals, as for each pulsation the time required varied, for instance, $26\frac{1}{5}$ sec. $24\frac{4}{5}$ sec. 45 sec. $25\frac{2}{5}$ sec. $49\frac{3}{5}$ sec. etc.

During the pulsation of the contractile vessel, a small quantity of blood from the efferent enters the afferent, because the blood vessels have no valves to prevent such reverse course.



Text Fig. 10. Illustration of the pulsation of the contractile vessel.

I then attempted to determine the direction of the blood flow by slowly injecting Fol's metagelatin mixed with Chinese ink from many portions of the blood vessels.

1) I injected this gelatine solution towards the afferent or towards the efferent vessels from the center of the contractile vessel and observed the entrance of the solution into the intestinal lacunæ of the posterior part of the small intestine by the pulsation of the contractile vessel. I always saw an entrance of a small quantity of the solution into the afferent vessel, but it never reached the rete-mirabile. Thus the gelatine reaches the lacunæ of the anterior intestine through the commissures and the diagonal vessel, and especially in the last vessel reaches quickly the beginning of the small intestine, and then comes back to the contractile vessel from the intestinal lacunæ and from the rete-mirabile. It took 30 to 40 min. to circulate.

2) Injecting into the first commissure near the posterior small intestine toward the anterior small intestine, I saw the stuff entered the lacunæ of the latter from the commissure, and, at the same time some enter the same lacunæ from the other commissures by way of the ventral side of the posterior small intestine and thus both parts of the intestine became black, at first the ventral then gradually, the dorsal side.

3) I injected from the anterior ventral blood vessel which lies under the stomach towards the circular blood vessel, and observed, in the first place, a large quantity of the stuff enter the lacunæ of the anterior small intestine, and then proceed to the lacunæ of the posterior small intestine, though only a small quantity of it took the opposite course towards the circular blood vessel.

4) The injection of the stuff into the diagonal blood vessel near the anterior small intestine in the direction of the posterior shows that it reached the posterior small intestine by way of the diagonal vessel and then the stuff proceeded to the anterior small intestine by way of the commissures.

The direction of the normal blood circulation is, as the above experiments show, perfectly definite, though on account of the absence of a valve, some small quantity of blood may sometimes take a reverse course. This normal direction just mentioned is that shown in Experiment 1.

A small quantity of the stuff which reached the ventral blood vessel, which lies on the wall of the peristomachal sinus, entered the peristomachal sinus and then the lacunæ of the reproductive organ by way of the lacunæ of the wall of peristomachal sinus, and finally arrived at the circular blood vessel.

The ink could not enter the dorsal vessel on the wall of peristomachal sinus on account of its very slender diameter, but it seems highly probable that the blood which comes from the ventral blood vessel enters the dorsal vessel, passing through the circular blood vessel. Because the diameter of the dorsal blood vessel is smaller than that of the ventral vessel, especially as it becomes smaller near the circular blood vessel, and this dorsal vessel communicates posteriorly with the afferent blood vessel, I consider that a part of the blood in the circular blood vessel, which comes from the ventral, runs posteriorly to the dorsal blood vessel and that this vessel becomes gradually larger by collecting the blood which comes along the wall of the peristomachal sinus from the ventral vessel, until, at last, the blood enters the afferent vessel.

From the fact that I many times saw the red corpuscles present in the peristomachal sinus and from the fact that the experiment shows that ink can enter there also, it seems probable that a small quantity of blood may be able to pass the peristomachal sinus, the peripharyngeal sinus, and the cœlo-peripharyngeal connection from the ventral blood vessel, which lies on the wall of peristomachal sinus, and may ultimately enter the cœlomic cavity, as these show anatomical connection.

We have shown in the experiments given that the stuff failed to go forward from the circular blood vessel on account of the presence of a large quantity of blood plasma, but from the anatomical point of view, the blood can enter the radial water canal through the radial blood vessels at the posterior extremities of the anal papillæ. (Text Fig. 3.) I believe the blood, though its amount may be small, normally goes to the radial water canal through the capillary network of the large intestine, since this network connects anteriorly with the ventral blood vessel and posteriorly with the radial water canal.

In a word, there are two blood circulations in *Caudina chilensis*, the one circulates around the visceral organs, and the other circulates to the perivisceral cavity from the cœlo-peripharyngeal connection and from the madreporite through the radial water canal and the circular water canal.

TEXT FIG. 11.

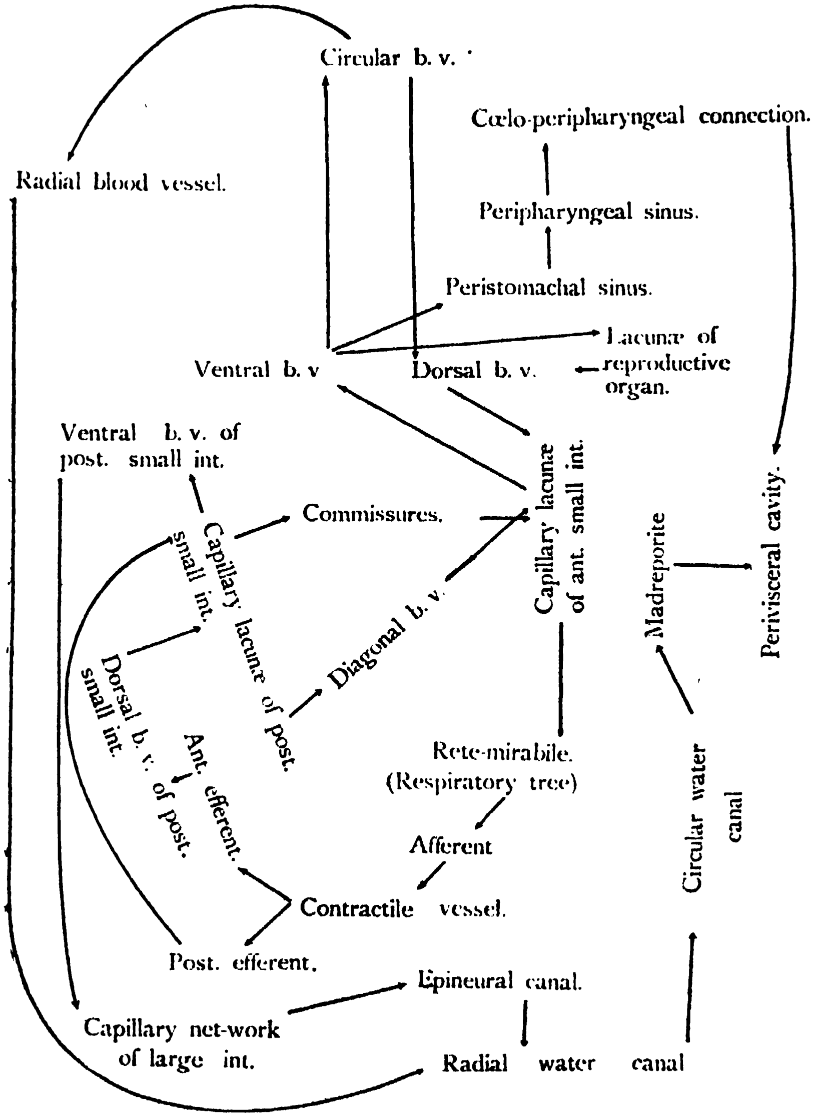


Diagram to illustrate the blood circulation of *Caudina chilensis*.

CONCLUSION.

From the above investigations, the relation between the perivisceral cavity, the blood, and the water vascular systems is shown diagrammatically in Text Fig. 11.

I consider it is very significant not only from phylogenetic standpoint, but also from physiological point of view, that the perivisceral cavity is continuous with the exterior by means of the five cœlo-anal canals which I have discovered.

Further careful physiological investigations are required to explain why the brown, the fusiform, and the crystal corpuscles are absent in the blood vessels, despite the fact that all kinds of corpuscles are present in the perivisceral cavity.

The reasons for the presence or the absence of some forms of red corpuscles in each of these three portions mentioned needs further investigation.

Before leaving the subject, my warmest thanks are due to Prof. S. HATAI, and Prof. S. HOZAWA for the valuable suggestions which I have received from them for this investigation, and I am also indepted to Assistant Prof. S. NOMURA and to Assistant Prof. S. KOKUBO for their kind advices during my stay at the Asamushi Marine Biological Station. I also wish to thank to Prof. M. TAHARA for his advice and suggestion while I was taking the photo-micrograph.

LITERATURE CITED.

1) MITSUKURI, K. 1912. Studies on Actinopodous Holothurioidea. Jour. Coll. Sci. Tokyo Imp. Univ. vol. 29.

2) GEROULD, J. H. 1896. Anatomy and histology of *Caudina arenata* GOULD. Bull Mus. Comp. Zool. vol. 29.

3) DENDY, A. 1898. On some points in the anatomy of *Caudina coriacea* HUTTON. Jour. Linn. Soc. London Zool. vol. 26.

4) KINDRED, J. E. 1926. A studies of the generic relationship of the "Amœbocytes with spherules" in *Arbacia*. Biol. Bull. Mar. Biol. Lab. vol. 50.

5) THÉEL, H. 1921. On amœbocytes and other cœlomic corpuscles in the perivisceral cavity of Echinoderms III., Holothurids. Ark. for Zool. Bd. 13.

6) HOWELL, W. H. 1886. Observations upon the blood of *Limulus*, *Polyphemus*, *Caltinectes*, *Hastatus* and a species of Holothurians Stud. from Biol. Lab. Johns Hopkins Univ., Baltimore. vol. 3.

7) GOODRICH, E. S. 1919. The pseudopodia of the leucocytes of invertebrates. Quat. Jour. Micro. Sci. vol. 64.

EXPLANATION OF PLATES.

PLATE 1.

General anatomy of *Caudina chilensis*.

A.	Anus.	Md.	Madreporite.
A. s. i.	Anterior small intestine.	Pa.	Pharynx.
a. e. V.	Anterior efferent vessel.	P. s. i.	Posterior small intestine
a. V.	Afferent vessel.	p. e. V.	Posterior efferent vessel.
c. R.	Calcareous ring.	P.	Polian vesicle.
c. b. V.	Circular blood vessel.	R. c. m.	Radial cloacal muscle.
Co.	Commissure.	R.	Rete-mirabile
d. B. V.	Diagonal blood vessel.	r. T.	Respiratory tree.
G. p.	Genital papilla.	St.	Stomach.
g. O.	Genital organ.	S	Stone canal.
L. m.	Longitudinal muscle.	Tl.	Tail.
L. i.	Large intestine.	T.	Tentacle.
M.	Mouth	t. A.	Tentacular ampulla.
Me.	Mesentery.	W.	Circular water canal.

PLATE 2.

Fig. 1. Longitudinal section through the buccal cavity, showing the cœlo-peripharyngeal connection. ×20.

Fig. 2. Cross section, the same. ×50.

Fig. 3. Section of the rete-mirabile showing the relation between it and the respiratory tree. ×20.

Fig. 4. Longitudinal section through the buccal region, showing the ambulacural system, etc. ×50.

Fig. 5. Longitudinal section through the posterior termination of the tail, showing the anal papilla and the cœlo-anal canal. ×30.

Fig. 6. The same, as Fig. 5, but especially showing the connection between the perivisceral cavity and the anal opening through the cœlo-anal canal. ×200.

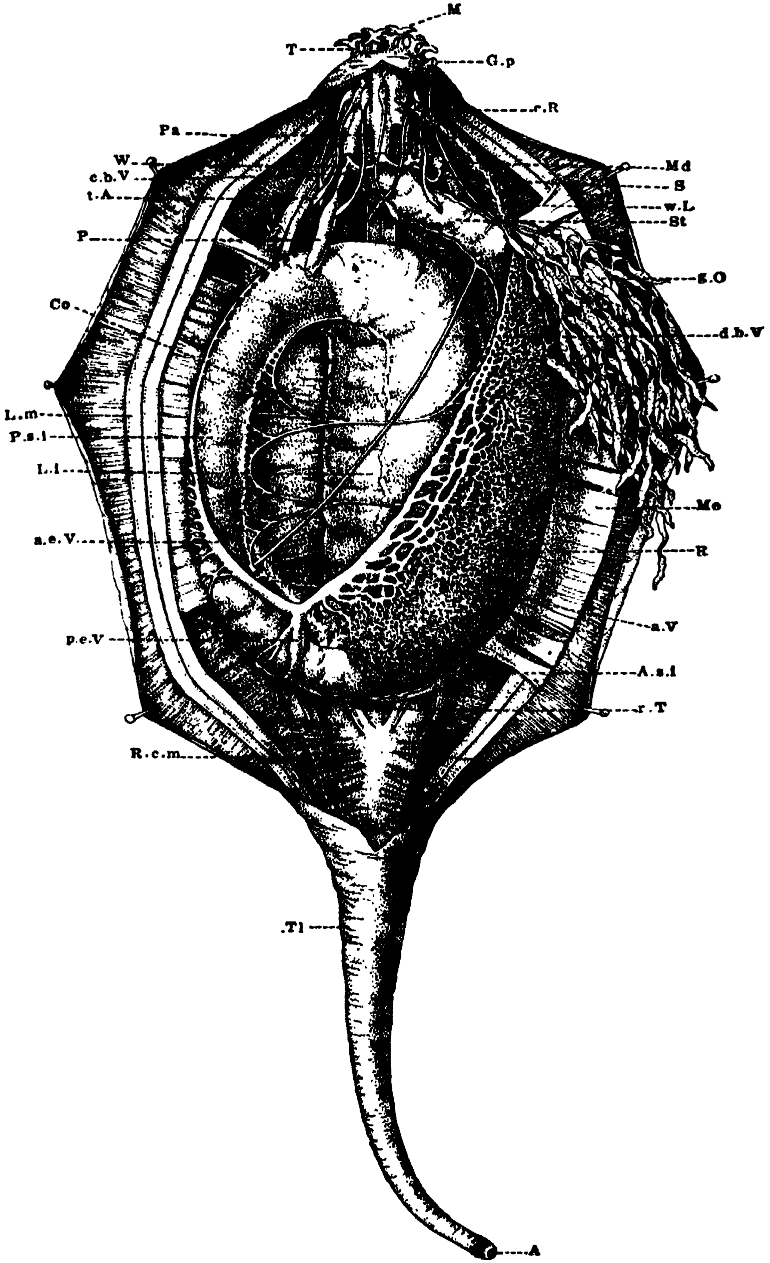
Epi.	Epithelium.	c. R.	Calcareous ring.
Spi.	Spicule.	T. Am.	Tentacular ampulla.
C. T.	Connective tissue.	P. P. S.	Peripharyngeal sinus.
h. n. C.	Hyponeural canal.	N. R.	Nerve ring.
e. n. C.	Epineural canal	R. M.	Rete-mirabile.
M.	Mouth.	Ph.	Pharynx.
r. W. C.	Radial water canal.	c. p. C.	Cœlo-peripharyngeal connection.
r. b. v.	Radial blood vessel	c. p. p. S.	Cœlo-peripharyngeal septum.
r. N.	Radial nerve.	c. a. C.	Cœlo-anal canal.

cir. M.	Circular muscle.	a. P.	Anal papillæ.
long. M.	Longitudinal muscle.	A.	Anus.
T.	Tentacle.	r. N. ex.	External band of radial nerve.
p. v. c.	Perivisceral cavity.	r. N. i.	Internal band of radial nerve.
r. T.	Respiratory tree.		

PLATE 3.

Fig. 1.	Fresh material from the perivisceral cavity.
Fig. 2.	Fixed and stained by May-Greenwald's method.
Fig. 3.	Fixed and stained by methyl-green-pyronin.
Fig. 4.	Fixed and stained by Giemsa's method.
Fig. 5.	Fixed and stained by alcoholic eosin solution.

a.	Red corpuscle.
b.	White corpuscle.
c.	Fusiform corpuscle.
d.	Brown corpuscle.
e.	Crystal corpuscle.
f.	Minute corpuscle.


M
T
G.p
r.R
Pa
W
Md
c.b.V
S
t.A
w.L
St
P
g.O
Co
d.b.V
L.m
P.s.l
L.l
Mo
a.e.V
R
a.V
p.e.V
A.s.l
r.T
R.c.m
Tl
A

SAKUMA del.

Fig. 1

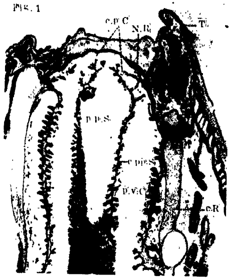


Fig. 2

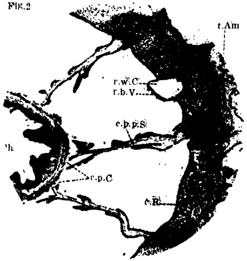


Fig. 3

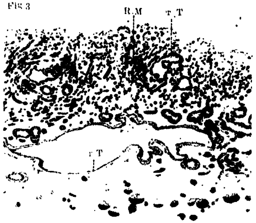


Fig. 4

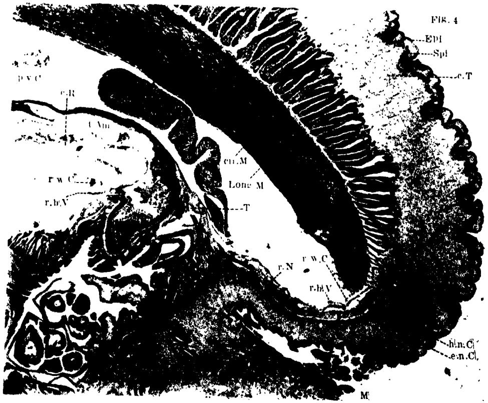


Fig. 5

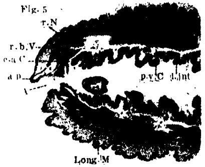
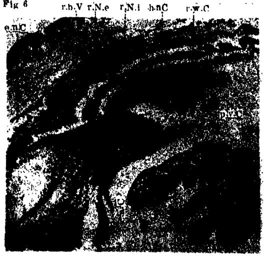


Fig 6



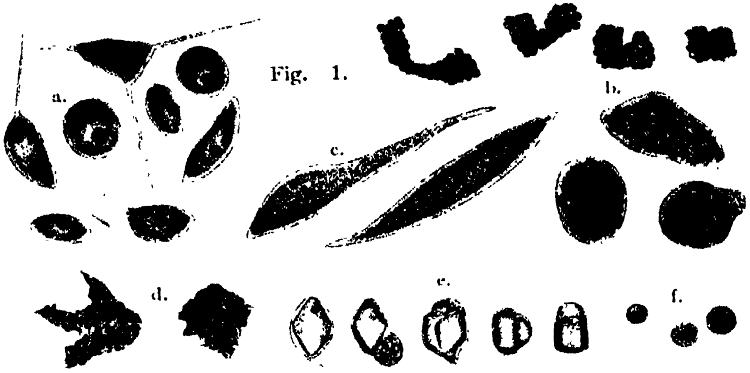


Fig. 1.

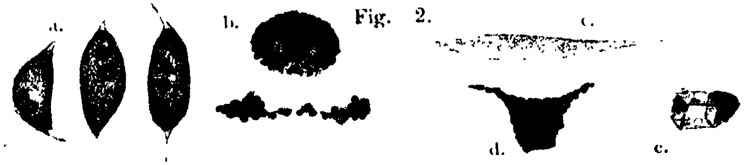


Fig. 2.

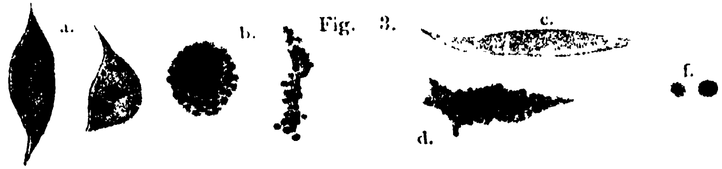


Fig. 3.

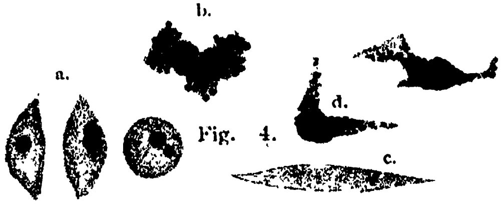


Fig. 4.

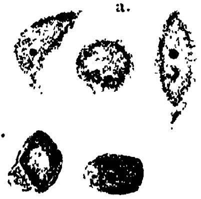


Fig. 5.

Physiological Characteristics of Caudina Muscle, with some Accounts on the Innervation.[1]

BY

LIEH TAO.

(Psychiatric Laboratory, Tokyo Imperial University.)

1. *The lengthening reaction, Loading contracture: Heat rigor and contractibility with regards to temperature: On the normal state of muscle in body.*
2. *Spontaneous rhythmical contraction.*
3. *Stimulation phenomena: Induction shocks, frequent induction stimulation; Latent time; Transmission of contraction; Constant current; On the factors which accelerate the relaxation; Fatigue phenomena and lengthening effects.*

BIEDERMANN[2] has investigated the muscle physiology of *Holothuria* and *Synapta*, especially their stimulation phenomena. JORDAN[3] examined mainly the lengthening reaction of *Holothuria* and *Stichopus* muscles and teguments with regard to the nervous influence upon them. The material used in the present investigation was *Caudina chilensis* MÜLLER, collected from a shallow beach near Asamushi Marine Biological Station. The five longitudinal muscles of *Caudina chilensis* are connected to the body wall by many fibrous membranes, which are parallel to each other but vertical to the axis of the longitudinal muscle. The circular muscle bands are attached directly to both sides of these membranes. The longitudinal muscles can be easily isolated, though circular muscle bands are difficult, and in the latter muscle the tegument always adheres to the sample. According to GEROULD,[4] the muscle of Caudina arenata has the typical structure of smooth muscle and the length of a single fiber, under ordinary conditions of relaxation, is stated to vary from 0.6 to 0.7 mm.

---

[1] A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

[2] W. BIEDERMANN, Zur Physiologie der glatten Muskeln, Pfl. Arch., Bd. 46, 1898, S. 398.

[3] H. JORDAN, Über reflexarme Tiere, IV. Holothurien, Mitt. I. Die Holothurien als hohlorganartige Tiere und die Tonusfunktion ihrer Muskulatur, Zool. Jahrb., Bd. 34, 1914; Mitt. II. Die Reizbarkeit und der Einfluss des zentralen Nervensystems auf die Muskulatur und die muskelähnlichen Fasern der Haut (auf Erregbarkeit und Tonusfunktion), Zool. Jahrb., Bd. 36, 1919, S. 109.

[4] J. H. GEROULD, The anatomy and histology of *Caudina arenata Gould*, Bulletin of the Museum of Comparative Zoology at Harvard College, Vol. 29, 1896, p. 124.

The tegument of *Caudina chilensis* has a total thickness of about 1.5 mm. in adult individuals, and thus this species belongs to the Holothuroidea having a thinner tegument. Whether this species possesses any "muskelähnliche Fasern der Holothurienhaut" in JORDAN's sense, is questionable, unless we admit the elastic fibers of the connective tissue to be muscle-like fibers. My preparations, which were stained with several modified VAN GIESON's methods, show the staining reaction of the connective tissue layers of Caudina and Stichopus, which differ clearly from the wall musculature, with the exception of the muscle cylinders, though both contractile and tonus muscles of bivalve molluscs show a similar staining reaction. The muscle cylinders in the connective tissue layer seem to play no part in the shortening or the lengthening of the tegument.

As it is technically difficult with Caudina to preserve the radial nerve intact when the tegument is removed from the muscle, the effect of the presence of the radial nerve upon the lengthening reaction of muscle, as well as the stimulation of the radial nerve, is not investigated.

I have not yet tested whether the tegument of Caudina possesses the characteristics of tonus muscle, as JORDAN and, recently, UEXKÜLL[1] tried to prove the presence of such a reaction on Holothuria and Stichopus tegument.

The central nervous system of Caudina, according to GEROULD's research on *Caudina arenata*, is composed of the nerve ring and five radial nerve bands, as other Holothuroidea. The outer band arises from the nerve ring, but the inner band disappears entirely, as a band, immediately posterior to the junction of the outer band with the nerve ring. Although it is difficult to determine the exact distribution of the peripheral nerves arising from the radial nerve bands, it can be distinctly seen that the inner band distributes fibers to the transverse and the longitudinal muscles of the body wall, whereas the outer band chiefly innervates the tegument. The nerve net system of Caudina consists of the sensory cells of the epithelium, subepithelial nerve plexus, and the layer of nervous tissue which is just above the transverse muscles of the body wall. GEROULD's observation on the connection of both systems is still obscure, except that there are isolated fibers from the tentacle nerves connected with the subepithelial ganglionic cells.

---

[1] J. v. UEXKÜLL, Die Sperrmuskulatur der Holothurien, Pfl. Arch., Bd. 212, 1926, S. 1.

As shown in the preceeding pages, from a physiological ground there must be nervous elements which possess the properties of transmission of contraction, of rhythmical contraction, and of acceleration upon relaxation. But which nervous elements are responsible in performing these various functions mentioned above can not be determined from the anatomical standpoint alone.

As a physiological solution, ordinary sea water was used. It was found that the various salts, as well as the osmotic pressure of sea water, differs but very slightly from those found in the body fluid of Caudina.[1] In sea water the contractibility of muscles remains unchanged for a period of 4–5 hours at least; besides no marked increase of the stimulus threshold was observed. We may anticipate in the future still better artificial physiological solutions based on the chemical composition of the body fluid.

The induction stimulation was applied in a muscle-trough, though at the time of stimulation the sea water was emptied out. For the monopolar constant current stimulation, a whole body of Caudina or an excised muscle was laid on cotton, as an indifferent pole. The cotton was saturated with sea water, and the object was stimulated by either a platinum electrode or an unpolarizable electrode. Constant current stimulation of excised muscle was accomplished with a fluid electrode, which is composed of a series: Zn, saturated solution of $ZnSO_4$, 5–10% KCl-gelatine, and sea water. The length of the muscle was measured for the most part by a lever and kymograph, and also by a horizontal telescope for correction. In Tables 1 and 2 the true length is given, others have a magnification of 4.6 times.

I wish to express here my sincere thanks to Prof. S. HATAI, who suggested the problem and under whose kind guidance and encouragement the present investigations were carried on at Asamushi Marine Biological Station during the summers of 1924 and 1925.

---

[1] K. OKAZAKI und T. KOIZUMI, Über die Leibshöhlenflüssigkeit von Holothurien, *Caudina chilensis.*, Science Reports of the Tohoku Imperial University, Vol. 2, 1926, p. 139.

I. The lengthening reaction.

Caudina muscle shows the general equilibrium reaction of an elastic body in the instants of loading as well as of unloading. Furthermore the lengthening reaction is characteristic of smooth muscles in the case of continual loading. The contracted Caudina muscle shows a very small amount of relaxation as long as it is left unloaded, though it begins to lengthen on the slightest loading.

For the lengthening reaction with reference to time, the following empirical formula is appropriate, as far as the initial equilibrium reaction and the loading contracture are exclusively concerned:

$$L = mt + p\left(1 - e^{-\frac{vt}{p}}\right),$$

where L, lengthening per unit length in cm; t, time in min.; and m, p, v are coefficients, v is a quantity concerning the initial rate of lengthening. The values of the coefficients were determined from the values given by any three appropriate points.

TABLE 1. (FIG. 5, d; Longitudinal muscle pair; middle portion; 25°C; p, 1; v, 0.077; m, 0.0074; load, 1.3 gms).

t	0	10	20	30	40	50	60	70	80	90	100
L (observed)	0	0.61	0.85	1.00	1.15	1.30	1.41	1.50	1.59	1.65	1.74
L (calculated)	0	0.61	0.93	1.14	1.25	1.35	1.43	1.51	1.59	1.67	1.74

For comparison, I tested the lengthening of frog muscle against loading, and found the same formula is suitable, putting $m=0$:

$$L = p\left(1 - e^{-\frac{vt}{p}}\right).$$

TABLE 2. (FIG. 1; Frog sartorius, excised; in RINGER's solution; 27°C; p, 0.115; v, 0.0076; m, 0; load, 2.6 gms).

t	0	10	20	30	40	50	60	70	80	90
L (observed)	0	0.054	0.087	0.098	0.108	0.108	0.110	0.114	0.114	0.114
L (calculated)	0	0.056	0.084	0.099	0.107	0.110	0.112	0.113	0.1144	0.1147

The values of the coefficients vary according to the initial "hardness" of the excised muscle, which depends on the duration of the stay in sea water before loading, and certain other factors. Consequently, the lengthening curve as a whole, with the exception of the loading contracture (see page 271), may be divided into the two forms: plastic form, having a greater value of *m* and a smaller *p*, *v*; and elastic form, having a smaller value of *m* and a rather greater *p*, *v*. The caudina muscle, in a state of rigor produced by means of either distilled water or heat, shows the complete elastic form of lengthening, as in the case of the skeletal muscle of the frog.

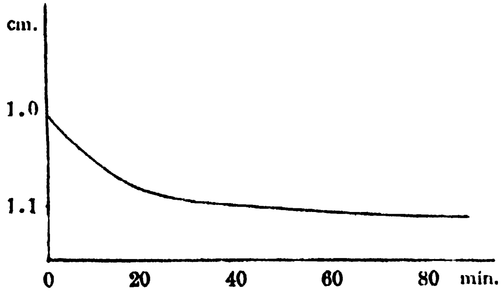


Fig. 1. Excised frog sartorius, load, 2.6 gms.

Caudina muscle gives a marked shortening as soon as it is put into distilled water, then it lengthens in complete elastic form, tending to a finite length. (FIG. 2) Such muscle, of which the lengthening rate became nearly equal to 0, if placed into the sea water again begins to lengthen anew, but tends to have another finite length (complete elastic form).

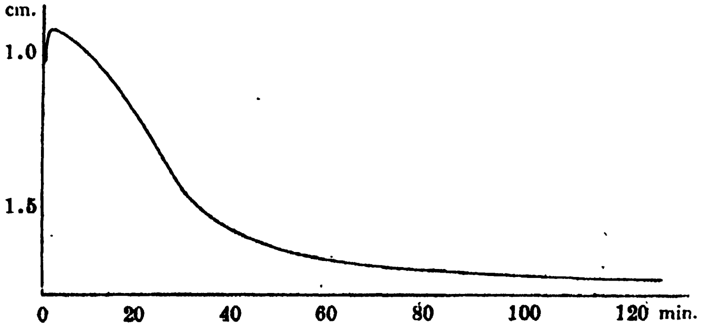


Fig. 2. Lengthening of Caudina muscle in distilled water; middle portion of right-ventral longitudinal muscle; load, 1.8 gms.

FIG. 3 shows four lengthening curves in which the muscle was loaded with weights 0.3, 1.3, 2.6, 3.9 gms. respectively from which it is obvious that the values of *m*, which represents approximate value of angles of asymptote, varies nearly proportionately with the given weight.

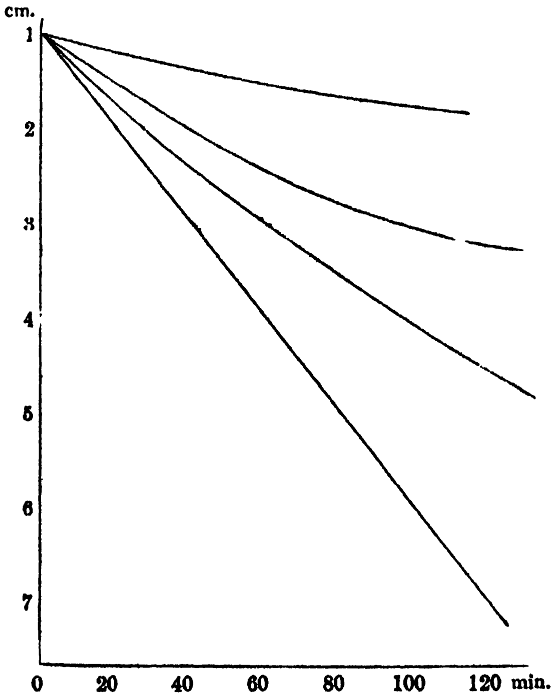


Fig. 3. Anterior and middle portion of mid-ventral longitudinal muscles.

During a lowering of temperature, Caudina muscle shortens as long as the cooling is in advance, but lengthens if the temperature stays constant.

The lengthening of Caudina tegument is approximately linear against continual loading and shows no tendency to reach a finite length. (FIG. 4) Under an equivalent loading, the longitudinal and the circular muscles with the tegument give nearly half the amount of lengthening as compared with the lengthening of muscle and tegument alone, and exhibit, in general, a more elastic form. It is conceivable from this fact that the amount, as well as the character, of the connective tissue contained in the muscle is influencing the lengthening reaction of the muscle.

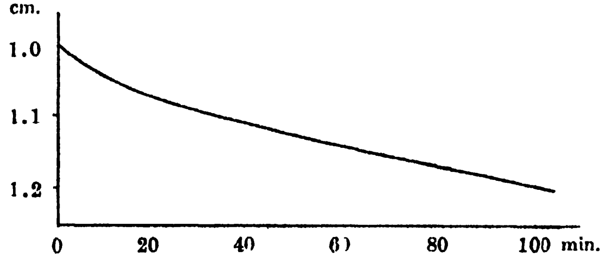


Fig. 4. Caudina tegument; lengthwise, radial band; load, 1.3 gms.

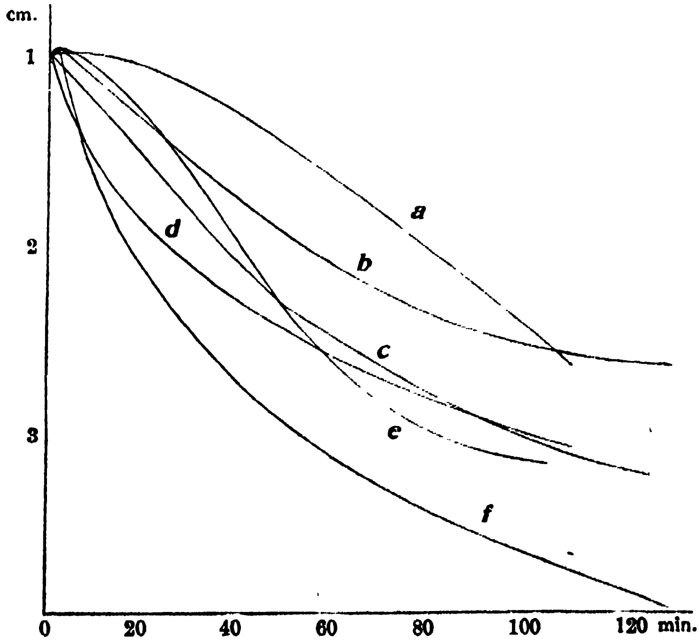


Fig. 5. Middle portions of different longitudinal muscles; *s*, sectional area in cm^2; load, 1.3 gms. S: *a*, 0.08; *b*, 0.08; *c*, 0.07; *d*, 0.06; *e*, 0.06; *f*, 0.05.

Loading contracture. Caudina muscle often shows a sort of contracture even after a quite gentle application of load, which is characterized by very slow rates and a small amount of contraction and relaxation as compared with the cases of mechanical or electrical stimulation, and at times the relaxation takes place very slowly so that the curve as a whole is concurve to the *L*-axis. (FIG. 5) This sort of contracture was briefly described by JORDAN (1919) on Holothuria muscle as a phenomenon of muscular nature under loading without radial nerve. This "loading contracture" mentioned above may also be considered as a muscular reaction against the "Dehnungsreiz" (*Straub*), which is a muscular reaction similar to the re-shortening due to unloading.

The loading contracture is prominent in muscle which was taken from the middle portion and from larger individuals. According to HOZAWA,[1] specules are found more abundantly in the middle portion of longitudinal muscles and decrease towards both terminals, and, furthermore these specules are not found in small individuals. According to these parallel facts, the presence of specules may be somehow related to the loading contracture in favoring conditions for such a muscular reaction, but to make a definite statement requires further investigation.

HEAT RIGOR AND CONTRACTIBILITY WITH REGARD TO TEMPERATURE.

The lengthening of the longitudinal muscle with increasing temperature ceases at about 40°C and turns into slow shortening, which is gradually intensified till it reaches 65°–70°C, where the shortening reaches maximum. Lengthening occurs no more after the temperature reaches 40°C. In this experiment the weight of the load was 1.2 gms.[2]

Caudina tegument (lengthwise) lengthens with increasing temperature but marked shortening takes place at 52°–54°C which process continues, as the temperature rises further. The circular muscle *with*

---

[1] Private communication.

[2] Such lengthening was considered as the effect of loading by various authors (BRODIE and RICHARDSON). VERNON considers it also as a physical phenomenon rather than as an inherent muscular property.

H. M. VERNON, Heat rigor in cold blooded animals, Jour. of Physiol., Vol. 24, 1899, p. 239.

tegument presents also a similar reaction at the same temperature. On the other hand, the longitudinal muscle does not show any marked shortening in the same range of temperature. Consequently this shortening may be taken as a characteristic of the tegument rather than of the muscle itself. In the neighbourhood of 65°-70°C, the tegument bends toward the circular muscle, indicating a shortening of the muscle and not the tegument. Therefore circular muscle differs in no way from longitudinal muscle in this regard.

Contractibility against induction stimulation disappears either below 5°C or above 40°C.

ON THE NORMAL STATE OF MUSCLES IN THE BODY.

BOVARD[1] has shown on earthworm that after cutting the nerve, the region immediately behind the cut showed an enlargement of the segments. There the circular muscles seemed to have lost their tone due to the cut to an extent of from 3 to 5 segments. This enlargement behind the cut region disappeared as the normal reactions returned.

I, however, obtained a reverse result on Caudina from a similar experiment in which I cut one or all (five) radial nerve regions through the body surface, being careful not to injure the muscles, and observed that there was a local contraction for several hours of both the circular and the longitudinal muscles within a range of 1.5-2 cm. posterior to the cut. If, however, only one nerve is cut, the contraction of the circular and the longitudinal muscles is limited to that locality. This local contraction soon becomes indistinguishable, owing to the gradually increasing contraction of the whole body, just as when Caudina receives a severe injury.

There must be, therefore, an important difference between earthworms, on one hand, and Caudina, on the other, concerning the normal state of the muscles, because, as these experiments show, the earthworm muscle in the normal state has a tonus due to the innervation, while the existence of such tonus is questionable in the Caudina muscle.

The cutting open of the body wall of Caudina is followed by the instantaneous relaxation of the entire body, which turns, at the next moment, to a contraction of the whole musculature. This apparent relaxation of the entire body is not associated with the relaxation of the muscle, but is due to a decrease of the internal pressure due to the sudden outflow of the body fluid. The contraction of the muscles occurred only after the passing of the necessary latent time, and therefore an ordinary tonus of the wall musculature is not necessarily to be taken into account in explaining this phenomenon of relaxation.

---

[1] J. F. BOVARD, The function of the giant fibers in earthworms, Univ. of California Publ. in Zool., Vol. 18, No. 8, 1918.

A probable explanation of the occurrance of contraction in the posterior region after the sectioning of the nerve seems to me to be that, normally, an excitative wave, which produces contraction, appears to be proceeding from the posterior to the anterior, which, however, in the resting state is inhibited by the nervous system, but when the nerve is cut, the contraction must follow as a necessary consequence. This hypothesis just stated is in favor of UEXKÜLL's theory in relation to the "reservoir of excitation" in the nervous systems of lower invertebrates. The direct type (PARKER) of locomotive movement of Caudina seems to support this view since in such a movement the motorial impulse proceeds from the posterior to the anterior, and the histological fact that the inner radial nerve has no direct connection with the nerve ring, but starts rather from the posterior extremity (GEROULD, 1896) adds further evidence favoring this view.

II. Spontaneous rhythmical contraction.

The rhythmical contraction, as far as the longitudinal direction of the whole body of Caudina is concerned, occurs in company with the

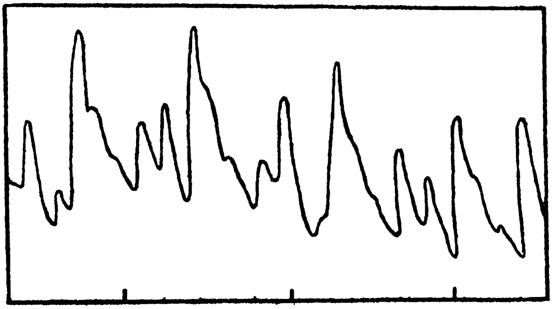


Fig. 6. Whole body; time signal, 1 min. ×4.6.

respiratory movement and is composed of an intensive contraction followed by several small contractions. (FIG. 6) The caudal end of Caudina, with its tegument and rectum, contracts rhythmically in quite the same way as the whole body. But if one slits it lengthwise on one side, it loses the small contractions and the regular strong contractions alone remain.[1] (FIG. 7.)

Circular muscle with tegument and cross sections of longitudinal muscles show pronounced rhythmical contraction. The alternation of large and small contractions (FIG. 8) is not a frequent phenomenon. If one removes from the above sample two of the sections of longitudinal muscles out of three, the rhythmical contraction still goes on; but the removal of the remaining one produces quite a feeble contraction or diminishes it totally.

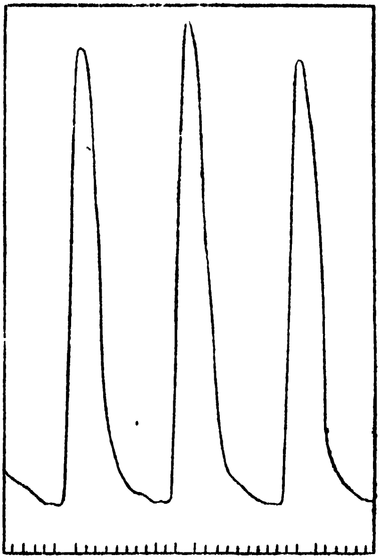


Fig. 7. Tail, slit lengthwise on one side; time signal, 5 sec. ×3.

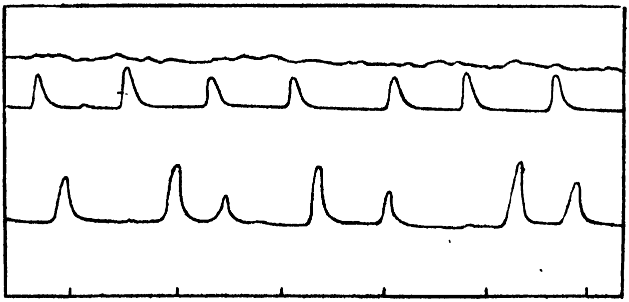


Fig. 8. Circular muscle with tegument and three sections of longitudinal muscles; in sea water; time signal 1 min.; load, 1.2 gms. ×3.

---

[1] A simple method to remove the effect of circular muscle used by BUDINGTON, but probably at the same time the effect of cloacal muscles is removed here.
R. A. BUDINGTON, Some physiological characteristics of Annelid muscle, Amer. Jour. of Physiol., Vol. 7, 1902, p. 155.

Longitudinal muscle if attached by tegument tissue shows, in the majority of cases, rhythmical contraction, otherwise there is scarcely any rhythmical contraction. It is to be noted that if there is present even a small fraction of tegument rhythmical contraction is produced. These facts seem to ascertain the neurogenic origin of the rhythmical contraction of Caudina muscle. But which nervous element is responsible here is not certain. Several experiments to test whether or not the radial nerve is in any way responsible for the above effect thus far have given no positive result. Neither the presence of the nerve ring nor its absence seems to influence this reaction.

The rhythmical contraction of the longitudinal muscle in the body fluid shows more irregularity and has a period of somewhat shorter duration (about 1 min.) than in sea water. (FIG. 9) In VAN'T HOFF's solution (26.9 gms. NaCl, 3.32 $MgCl_3$, 2.04 $MgSO_4$, 0.73 KCl, 0.51 $CaCl_2$, trace of $NaHCO_3$ and of K_2HPO_4 in 1 L.), the rhythmical contraction continues quite regularly for a long time. (FIG. 10) In HATAI's solution (23.2 gms. NaCl, 1.27 $MgCl_3$, 0.66 $CaCl_2$, 0.54 KCl, 0.008 NaH_2PO_4, 0.005 $NaHCO_3$ in 1 L.), longitudinal muscles with or without (apparently) tegument showed irregular, feeble contractions having no distinct rhythm. The presence of plenty of oxygen in the medium seems to favor an active rhythmical contraction.

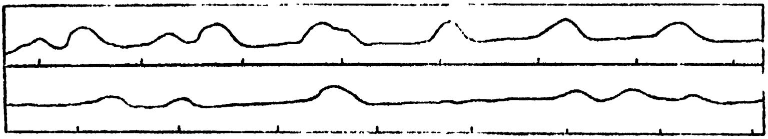


Fig. 9. Longitudinal muscle with a piece of tegument; in body fluid; time signal, 1 min.; load, 1.2 gms. 23–23.5°C. × 3.

Loading is a necessary condition for rhythmical contraction because it makes relaxation possible; besides it may have some accelerating influence, direct or indirect, upon the nervous element.

The rhythmical contraction of Caudina muscle begins in a moderate state of lengthening. The muscle which is rhythmically contracting shows, in general, a lengthening curve of elastic form. It is obvious from Table 3, that the ratio between the contraction height and the total length of muscle at the same instant, increases nearly proportionately with an increasing period which is also nearly equivalent to the increase of the muscle lengthening.

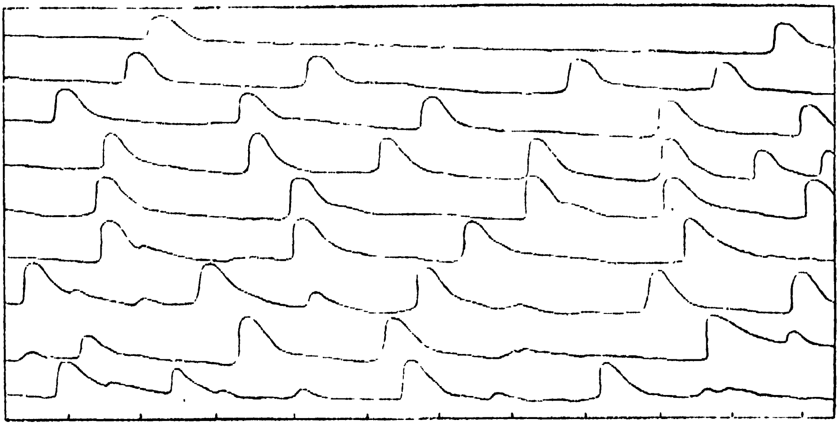


Fig. 10. Longitudinal muscle with a piece of tegument and nerve ring; in VAN'T HOFF's solution; time signal 1 min.; weight, 1.2 gms.; 23-24°C. ×2.3.

It is noticeable that, notwithstanding various conditions and the high degree of individual variations in respect to the stimulus threshold the deviation of the period is within a narrow range of 0.5–3.5 minutes. The same is true of the contraction height. In the longer muscle sample the whole length does not participate in the production of the rhythmical contraction but only a certain portion of it, i. e. that in the neighbourhood where the fraction of tegument is attached.

TABLE 3. (No. 5 in VAN'T HOFF's solution, others in sea water; 26–27.5°C; time in minutes).

No.	Initial length	Deviation range of intervals	Mean interval	(Shortened state)			(Lengthened state)		
				Height	Total length	Ratio (%)	Height	Total length	Ratio
1 circ.	1.5	0.5–1.5	1.0	0.07	2.0	3.2	0.28	2.1	13.0
2 ,,	2.1	0.5–2.5	1.8	0.02	2.3	0.9	0.22	2.7	8.0
3 ,,	1.4	1.0–3.5	2.0	—	—	—	—	—	—
4 long.	1.0	1.0–2.5	1.6	0.03	2.3	1.4	0.14	3.0	4.6
5 ,,	—	1.0–3.5	2.1	0.17	—	—	0.34	—	—

After the regular rhythmical contraction has commenced, the interval between the contractions is reduced gradually and afterwards becomes somewhat constant, for example: (time in minutes)

No. 3. 6.7 5.0 4.8 3.7 2.9 1.7....
No. 5. 8.2 2.4 3.5 2.0 2.8 2.4....

The rhythmical contractions with the greater deviation range of intervals tend to maintain such a relation for a longer period and are also associated with a greater mean interval and vice versa.

The rhythmical contraction generally commences with irregular small contractions, which are gradually polymerized into regular ones, but when the tegument tissue is quite small, the contractions of muscle are isolated from the beginning and are regular in form.

That Caudina muscle seems to have no measurable refractory period, like other smooth muscle, and that the interval of the initial regular contractions gradually decreases, seem to favor the storage process theory of rhythmical contraction in this species. The storage process theory being possible, under the influence of the nervous system, the nervous system must be able to show a large range of the tonus state. The possession of such a character just stated is conceivable from the functions of the primitive nervous system, as well as from that of the vegetative nervous systems of higher animals. If such an idea is admissible, then the functions of these two kinds of nervous systems, primitive and vegetative, should be identical in regard to rhythmical contraction. Indeed, in reality seme fundamental similarities are shown by voluntary and involuntary muscles. Involuntary muscle, whether striated or smooth, which is innervated by the vegetative nervous system, is spontaneously contractile, while voluntary muscle, which is innervated by both the vegetative and the cerebral nervous systems does not possess that ability.

III. Stimulation phenomena.

INDUCTION SHOCKS.

Caudina muscle reacts to electrical and mechanical stimuli with a contraction which is transmitted the full length, provided the tegument is completely attached to the muscle. (See page 281) However, the characteristic enlargement of the muscle, which occurs as the result of an intensified stimulus, is limited to the neighbourhood of the stimulation. The contractions do not show a definite maximal height. The summation is complete and always occurs if the secondary stimuli are given at any period between the latent and the contraction period.

A rapid form of contraction, produced by intensified stimulus, which BUDINGTON (1902), who applied the after-loading method, has noticed in Annelid muscle, was not observed in Caudina muscle. However, various forms of contractions according to several factors were noted. The rate of relaxation is somewhat greater when it takes place in sea water than in air. (Table 4) Both the contraction-relaxation time ratio and also the height-duration ratio are greater in the lengthened state than in the shortened state. (Table 5) The rate of contraction is slightly greater in muscle without tegument (FIG. 11, b, c), and the rate of relaxtion is markedly greater in muscle with tegument (FIG. 11, a, d, e; Table 6). The rate of relaxtion is much greater in the multiple induction stimulation than in the single induction stimulation. Comparison of Tables 5 and 6 makes this relation clear. The same situation holds as well for longitudinal muscle without tegument.

TABLE 4. (Longitudinal muscle with tegument; a single opening shock; shortened state; *c*, contraction time; *r*, relaxation time, the contraction remainder being neglected.)

	c	*r*
Relaxation in sea water	8″	6′15″
in air	7″	7′29″

TABLE 5. (Longitudinal muscle with tegument; several induction shocks; *h/d*, height-duration ratio.)

	c	*r*	*h/d*
Shortened state (relaxation in sea water)	7″	2′ 8″	1/11
Lengthened state (relaxation in air)	5″	1′17″	1/2

TABLE 6. (Average of several cases; longitudinal muscle; single induction shocks; loading, 1.2 gms. relaxation in sea water; lengthened state.)

	c	r
Muscle only	6.5″	4′30″
With tegument	7.0″	1′30″

Frequent induction stimulation. The frequency of induction shocks, which is necessary to arouse a continual tetanus-like contraction on Caudina muscle, is 1.7–2 per second. It is difficult to obtain a complete plateau. In most cases the height gradually decreases or becomes irregular though the stimulation goes on. This frequency of stimulation mentioned above belongs to a group of lowest value among other smooth muscles, and comes near to the case of cat's bladder (1 per second)[1]; also it is interesting to note that Caudina muscle is similar to that of cat's bladder in showing a higher value of latent time.

Induction stimulation of a frequency of 100 per second always calls forth a marked contraction at the beginning, but what succeeds it depends on the state of elongation of the muscle. In a shortened state longitudinal muscle (with or without tegument) soon commences a steep relaxation to such an extent that it often exceeds its initial length, and continues its rate of relaxation even after the stimulation is over. In the lengthened state, the behavior is variable but, in general, the muscle tends to preserve its contraction and shows a distinct relaxation as soon as the stimulation ceases. The rates of relaxation of the muscles through frequent induction stimulation are nearly equal, in both those with and those without tegument.

LATENT TIME.

The latent time of Caudina muscle against direct induction or constant current stimulation has a large range of variation and its distribution is shown in the following table;

---

[1] C. C. STEWART, Mammalian smooth muscle—cat's bladder, Amer. Jour. of Physiol., Vol. 4, 1900, p. 185.

TABLE 7. (20-23°C ; single induction stimulation)

Nos. of cases	Time in 1/10 sec.	0.7 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 12
	Anterior	0	3	1	3	4	1	3	2	0	1	1
	Middle	1	0	2	5	3	0	0	0	0	0	0
	Posterior	0	2	3	8	4	4	0	1	0	0	1
	With tegument	0	0	0	0	2	1	0	0	1	1	1
	Sum	1	5	6	16	13	6	3	3	1	2	3

The most frequent value is 0.3 second, which is about the same as the value given by the entire average. Such a great variation may depend to some extent on the difficulty in determining the initial point of contraction, due chiefly to the slowness of the contraction. However, a great variation of the state of reactivity seems to be present, as a physiological characteristic of less differentiated nerve muscle systems.

TRANSMISSION OF CONTRACTION.

If a longitudinal muscle preparation with tegument is fastened in the middle and stimulated at one end with a single induction shock, then the other end also shows a contraction. (FIG. 11, a) But in a similar preparation, if the tegument was not attached, the stimulation of one end produces only a mere trace of a contraction at the other end. (FIG. 11, b, c) Again, if the muscle is separated by a cut in the middle and the two portions are connected by the tegument, the stimulation applied to one end does not produce a contraction of the other portion ; in other words, the contraction wave is not transmitted through the tegument. (FIG. 11, d) If, however, the muscle and the tegument maintain a normal and natural continuity at both ends but in the middle the connection is separated about 5 mm., the transmission is very feeble. (FIG. 11, e) The same is true when the radial nerve band, which runs along the middle line between the tegument and the longitudinal muscle remains intact and both sides of the band are separated to the extent of about 5 mm. In the above experiments the middle portion of the muscle is necessarily injured but there is no interference in the transmission of contraction. Therefore, a necessary condition for ordinary transmission of contraction is the connection between the muscle and the tegument, most probably the presence of some nervous connection.

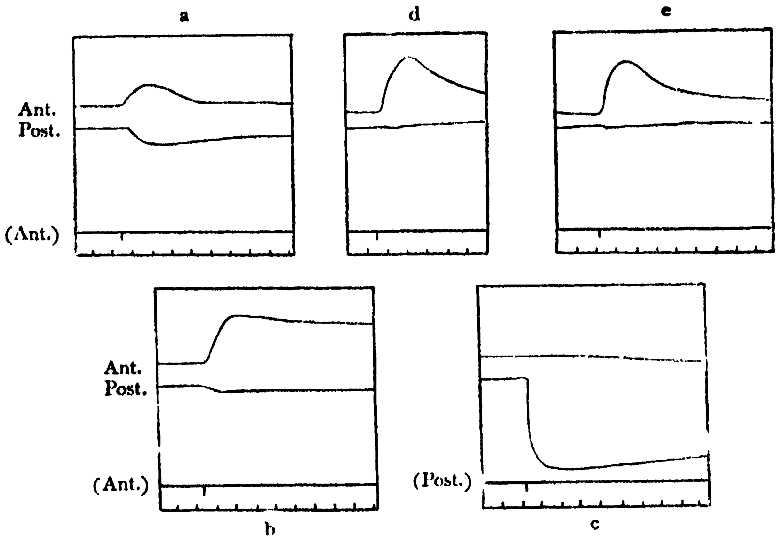


Fig. 11. Time signal, 5 sec.; (ant.) or (post.), close to the stimulation signal line, denotes the portion which was stimulated. ×3.

A feeble or moderate stimulus given at a definite point on the body surface produces a contraction of the circular muscle in the immediate neighbourhood of the interradial band, but when the stimulus is fairly strong, the circular muscles in the neighbouring interradial bands of the same latitude also show contraction. Although the phenomenon can be explained by a great decrement of the transmitting nervous element, I believe that an effect of direct extension-stimulus is more probable in this instance.

About the distant transmission, according to YAMANOUCHI's[1] observation, the tail of Caudina calls forth a reaction of the tentacles if it is cut within the anterior half, showing a latent time which is macroscopically unmeasurable. On the contrary, stimulation on the tentacles does not call forth any immediate contraction of the tail. These facts seem to indicate the probable presence of long sensory fibers, but not of long motor fibers.

---

[1] Private communication.

CONSTANT CURRENT.

When Caudina is stimulated on the body surface, the longitudinal muscle contracts more noticeably upon cathode stimulation but with the circular muscle anode stimulation produces a more obvious contraction. This observation agrees with that of BIEDERMANN (1898) on the muscles of *Holothuria* and *Synapta*, and confirms his explanation that upon monopolar anode stimulation, total contraction is more marked, though upon cathode stimulation the contraction is limited to the pole region, showing that PFLÜGER's law holds perfectly, in the sense that the excitation proceeds from the region where the negative charge enters or increases. It is more clearly seen in experiments on excised muscles under loading that there is no difference between the two kinds of muscles.

Dilatation is observed immediately under the pole in the case of monopolar anode stimulation[1] and most favorably with the unpolarizable electrode in contact with the surface of body. The opening contraction is difficult to be observed unless the muscle is loaded.

When excised Caudina muscles are stimulated with a constant current of a short duration in a fluid electrode, the rate of relaxation is generally greater than that of the induction stimulation.

If a slight contraction remainder is neglected, the ascending and descending branches of the graph are nearly symmetrical, which relation is not greatly affected by the strength of the current. A given strength of the current seems to produce a definite maximal height, but if the circuit is opened before the maximum is reached, further contraction ceases at once and relaxation takes place a few seconds later. (FIG. 12) For example, the contraction height of a curve produced by a duration of current for 0.2 second is about 1/4 of the maximum contraction produced by a duration of an equal current for 2.1 seconds.

Stimulation of muscles by means of a series caused by the closing and opening of constant current (the interval being about 1/40 sec.) gives a larger height-duration ratio, i. e. greater values of both rates of contraction and relaxation and a surpassing symmetry of the curve, when compared with a single closing and opening of an equal duration.

---

[1] M. FÜRST, Zur Physiologie der glatten Muskeln, Pfl. Arch., Bd. 46, 1898, S. 267.

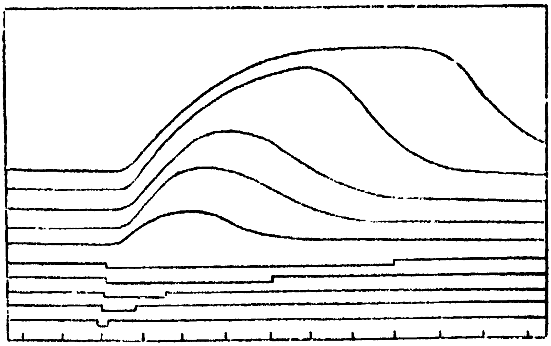


Fig. 12. Constant current; fluid electrode; longitudinal muscle in lengthened state; time signal, 1 sec. ×3.

TABLE 8.

	c	*r*	*h*	*d*
Single	2.2	5.2	0.8	8.5
Multiple (6/sec.)	3.4	6.1	2.3	9.8
Multiple (22/sec.)	5.2	6.2	2.9	11.8

As FIGS. 12 and 13 show, in the lengthened state the accelerating rate of the relaxation grows in proportion with the increased duration of the current and, similarly, the height of the contraction curve increases under the same experimental conditions, and in consequence, the contraction graph is composed of prolonged ascending and accelerated descending branches as the duration of the current is prolonged.

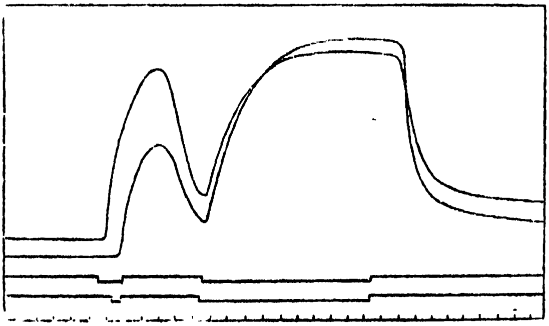


Fig. 13. Two contraction curves of succeeding constant current stimulation, with equal interval but different primary duration; conditions same as in fig. 12 ×3.

When the duration of the current is prolonged, the muscle in the shortened state relaxes, after a slight closing contraction, far beyond its previous length, and shows a noticeable opening re-shortening. In association with the degree of lengthening, the closing contraction increases proportionally, while the rate of relaxation during the flow of the current increases slightly in the somewhat lengthened muscle, and decreases in the greatly lengthened muscle. The muscle in the lengthened state maintains a certain degree of contraction during the flow of the current. The re-shortening due to opening gradually diminishes in reverse proportion to the degree of lengthening and finally disappears, till, in the considerably lengthened state, the muscle shows a distinct relaxation after opening. (FIG. 14, 15)

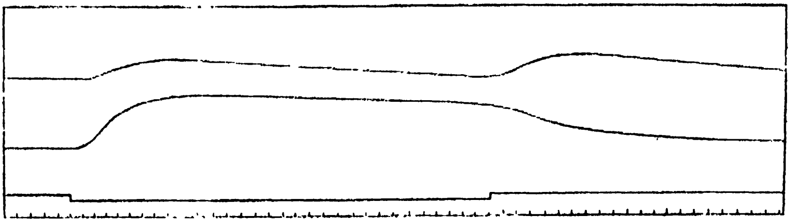


Fig. 14. Above, shortened state; below, lengthened state; conditions same as in fig. 12. ×2.3.

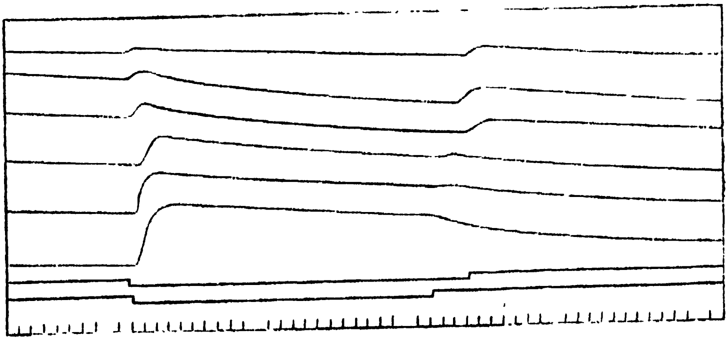


Fig. 15. Various degree of lengthening from above downward; time signal, 5 sec. ×3.

The shortening due to the opening is observed only when the relaxation during the flow of current has taken place to some extent.

That the height of the opening effect is approximately the same to the relaxed amount during the flow of the current, and that it has an appearance of re-shortening rather than of ordinary contraction seem to indicate that the relaxation during the flow of the current is a forced phenomenon and that the opening acts only as a removal of that force. This forced phenomenon is expressed by DE ZILWA[1] as relaxation "through diminution of elasticity." That the relaxation after this re-shortening is not accelerated by the presence of a tegument supports this view. The fact that the tegument is not responsible for the phenomenon just stated tempts one to suppose that this relaxation effect may be an intramuscular process which is called forth by the constant current, such as a polarized state of muscle cells shows. But whether there is no opening excitation at all is a question.

The relaxing effect of a constant current appears only when the current is strong enough to call forth the closing effect. BUDINGTON's relaxation phenomenon, i. e. the relaxation after contraction due to induction stimulation is accelerated by a feeble constant current, may be taken as a special case of the effect of this current.

The relaxation during the current and the re-shortening due to opening are very noticeable when longitudinal muscle with tegument and platinum electrodes are used. (FIG. 16)

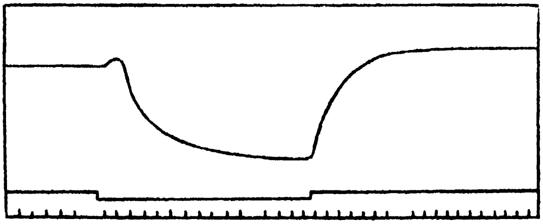


Fig. 16. Longitudinal muscle with tegument; platinum electrode; time signal, 5 sec. ×3.

ON FACTORS WHICH ACCELERATE RELAXATION.

Summarizing the results concerning the rate of relaxation, we find:

1) Contraction due to a single induction shock is followed by a

---

[1] L. A. E. DE ZILWA, Some contributions to the physiology of unstriated muscle, Jour. of Physiol., Vol. 27, 1901, p. 200.

prolonged relaxation; the presence of a tegument increases the rate of relaxation.

2) The muscle reacts to multiple induction stimulation in a form with large *c-r* ratio and *h-d* ratio; and it is indifferent whether the tegument is present or not.

3) The same relation holds for frequent induction stimulation; and also for series of stimulation caused by the closing and opening of the constant current.

These facts may be more concisely stated:

a) Relaxation is accelerated by multiple stimulation.

b) Relaxation is accelerated by the co-existence of the tegument with the muscle.

The former mechanism (a) must be an attribute of the muscle itself, and can be also expressed as: the relaxation depends on the degree of contraction or the amount of excitation. The latter mechanism (b) is most naturally considered a function of a certain nervous element which is active when the connection between the tegument and the muscle is complete, and may be explained either by a direct acceleration of relaxation or an ability to give multiple excitations to muscle from a single stimulus.

On the other hand, the rate of relaxation depends on the state of lengthening of the muscle. A relaxation, notwithstanding continual stimulation of both induction shocks and constant current, occurs only in a shortened state.

The relaxation during the flow of the current is considered to be a forced phenomenon, as mentioned in a previous page (286) but in the case of frequent induction stimulation a somewhat different interpretation must be made concerning the relaxation phenomenon, as there is no re-shortening due to opening.[1] This phenomenon may occur under certain quantitatively different conditions of the shortened state which produce a relaxation by skipping a contraction period, in the case of continuous stimulation. The same condition might favor the occurrence of forced relaxation due to constant current.

---

[1] Although MISLAWSKY found an opening contraction following frequent induction stimulation, in this case the phenomenon was proved to be of nervous influence, while in the present case the muscle reaction alone is shown. It must be added, however, that whether the nervous influence can produce a similar reaction in the case of Caudina is still to be investigated.

N. MISLAWSKY, Über die rhythmische Reizung der glatten Muskeln, Ztsch. f. d. allg. Physiol., Bd. 6, 1907, S. 1.

FATIGUE PHENOMENA AND LENGTHENING EFFECTS.

The lengthening of the muscle under continuous loading involves two effects, lengthening and fatigue effects, and the two factors must be distinguished from each other. Investigators on fatigue phenomena of smooth muscles often misunderstand these two factors as one and consequently the two features are taken for fatigue effect alone.[1]

Since the rhythmical contraction has a difinite pause between each contraction, it may be considered as a fatigueless process, if such experiment is not continued long. If such statement just mentioned is admissible, the changes in form of the contraction curve must be due to the lengthening effect of muscle and, in fact, we noted that the height of the curve of rhythmical contraction increases as the lengthening of muscle increases, and the increase of both the *c-r* ratio and the *h-d* ratio belong to the lengthening effect alone.

In the initial period of this lengthening effect the increase of the contraction height is conceivable as the sort of phenomenon which is shown by striated muscle in the case of repeated stimulation. This phenomenon just mentioned is, however, limited to the several initial contractions, while the lengthening effect lasts much longer. Therefore, the former can not be considered as affecting the latter except for the several initial contractions.

These lengthening effects are more distinct in preparations with a low stimulus threshold, which show less fatigue effect. However, with repeated stimulation the contraction remainder evidently becomes associated with a slight elongation of the relaxation time. With a high stimulus threshold, if the stimulus is repeated, the height of the contraction curve not only decreases very quickly, but the times of contraction and relaxation are markedly prolonged. These phenomena should be considered fatigue effects.

---

[1] "Pertaining to STRAUB's suggestion that the muscle curves with sharp apices may be due to a fatigued condition of the muscle, it should be said here, that, while preparations showing very slow relaxation when fresh, often come to relax much more quickly after having made a number of contractions, and thus give the sharp curves, yet the opposite is as often true also." (BUDINGTON, 1902)

As the fatigue effect and the lengthening effect are thus contradictory to each other concerning the height of the contraction curve and the rate of relaxation, the fatigue effect could be entirely masked by the latter in preparations with a low threshold, and, on the contrary, with a high threshold the lengthening effect is masked by the fatigue effect. Thus, two types of variation are possible in the contraction form in the course of stimulation, depending on whether the fatigue effect or the lengthening effect predominates.

The "temporary recovery" (BUDINGTON) of the height of the curve on reversing the direction of the current in the case of repeated constant current stimulations of short duration, is seen also with Caudina muscle under the same experimental conditions, using a fluid electrode. (FIG. 17)

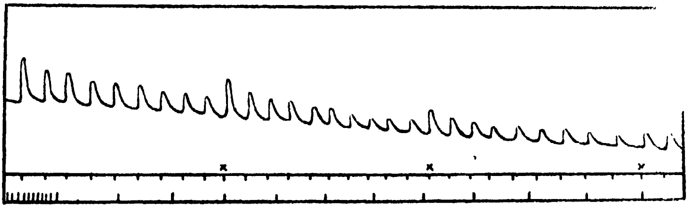


Fig. 17. The current direction was altered at the cross; time signal, 5 sec. ×3.

BUDINGTON, in his investigation on Annelid muscle, objects to ENGELMANN, who explains the temporary recovery based on the assumption that different parts of the muscle contract on the reversal of the direction of the current and maintains that the temporary recovery is due to an intracellular polarisation during the passage of a constant current. My own result supports BUDINGTON's view, since the fluid electrode which I have employed evokes excitation at the same portion by both directions of current. But if we take PFLÜGER's law into consideration, then the intracellular polarisation, if it occurs, must also mean that the excitation began at both different terminals (or cell membranes) by reversing the direction of current. Therefore, if ENGELMANN's statement of the different parts of muscle can be interpreted as the intracellular phenomenon, then his view becomes equally applicable to the phenomenon of temporary recovery as BUDINGTON's. Then the idea that fatigue occurs at the cell terminal or membrane agrees to the well established theory of nerve physiology that the fatigue of the nervous system is located in the synaptic membrane.

Summary.

Caudina muscle shows an equilibrium reaction and a lengthening reaction under loading, the latter is expressed satisfactorily by the following empirical formula;

$$L = mt + p\left(1 - e^{-\frac{vt}{p}}\right),$$

where L, lengthening; t, time; m, p, v, coefficients. As far as my observation goes, the middle portion of longitudinal muscles show, in the majority of cases, the *loading contracture.* Muscles which were treated previously with either distilled water or with heat give lengthening curves of complete elastic form, i. e. instead of the lengthening continuing indefinitely, it reaches a finite length.

Cutting of the radial nerve band produces a local contraction posterior to the cut.

The lengthening process of Caudina muscle stops at about 40°C, and is followed by a gradual shortening, which, in turn, shows a remarkable shortening at 65–70°C. Caudina tegument shortens at the temperature of 52–54°C. The range of contractibility against stimulation lies between about 5°C and 40°C.

Circular muscle behaves similarly to longitudinal muscle in the heat reaction as well as in the stimulation phenomenon.

Excised Caudina muscle contracts rhythmically in sea water or in VAN'T HOFF's solution, under loading; the period varies within a limit between 0.5–3.5 minutes. The presence of some attachment of tegument tissue seems to be a necessary condition for the occurrence of the rhythmical contraction, which suggests a participation of a certain nervous element in this phenomenon.

The connection between the tegument and muscle is a necessary condition for the transmission of contraction, though a transmission of contraction is often noticed with muscle without tegument, owing to the extension-stimulus.

In the case of direct stimulation, the latent time is most frequently 0.[illegible] sec. at 20–23°C. The necessary frequency of induction shocks

necessary to produce a tetanus-like contraction, though usually imperfect, is 1.7–2 per second.

The rate of relaxation is accelerated, among other factors, by multiple stimulation and also when the muscle is attached with tegument.

The response of Caudina muscle to continual stimulation differs according to its state of lengthening; in the shortened state the muscle soon turns to relaxation after an initial contraction, and in the lengthened state the muscle maintains its contraction state to a certain extent during the stimulation. This statement holds for both constant current and frequent induction stimulation. The nature of relaxation in the shortened state mentioned above is considered to differ according to the nature of the electrical stimulation (see page 287), and for instance, the re-shortening due to opening occurs only with the constant current stimulation.

A given strength of a constant current seems to call forth a definite contraction height, but in the muscle in the lengthened state the contraction is followed by relaxation as soon as the circuit is opened, before the maximum is reached.

Since the two opposing factors of fatigue aud lengthening co-exist in the case of repeated stimulation on loaded muscle, these two factors must be taken into consideration in interpreting the results of such experimentation.

Bastardierung als eine Ursache für die Entstehung der Chromosomenpolyploidie.

I. Bastard zwischen *Chrysanthemum marginatum* und *C. lavandulaefolium*.

Von

MASATO TAHARA u. NAOMASA SHIMOTOMAI.

Durch viele Arbeiten von verschiedenen Forschern ist es jetzt ganz klar geworden, dass Polyploidie der Chromosomen wenigstens im Pflanzenreich als ganz allgemeine Erscheinung anzusehen ist (TISCHLER, 1910; TAHARA, 1915; TÄCKHOLM, 1922; u. s. w.). Nun drängt sich uns die Frage auf, wie diese Verhältnisse bei so vielen Pflanzengruppen entstanden sind. Die im Jahre 1917 von WINGE zur Erklärung hierfür vorgeschlagene Bastardhypothese scheint neuerdings merklich an Bedeutung zugenommen zu haben, indem Kreuzungsversuche bei verschiedenen Pflanzen seitens verschiedener Autoren eine kräftige Stütze für diese Hypothese gebracht haben (DIGBY, 1912; BREMER, 1913; CLAUSEN and GOODSPEED, 1925; TSCHERMAK und BLEIER, 1926; CLAUSEN, 1926). Durch diese Untersuchungen wurde sicher festgestellt, dass Bastardierung unter nahe verwandten Arten manchmal Zunahme der Chromosomenzahl verursacht. Aber die Resultate lassen noch viel zu wünschen übrig, weil wir gegenwärtig nur erst wenig darüber wissen, wo und wie diese Steigerung der Chromosomenzahl beim Bartarde stattgefunden hat. Betreffs dieses Problems veröffentlichte ROSENBERG (1926) kürzlich einen Aufsatz, worin er zu begründen versucht, dass die Dyadenbildung mit diploider Chromosomenzahl bei den heterotypischen Teilungen der Bastardpflanzen als Hauptursache für die Vermehrung der Chromosomenzahl zu betrachten sein dürfte.

Beim Versuche an *Viola*-Bastarden kam CLAUSEN (1926) aber zu einem etwas anderen Resultat. Er schreibt: bei heterotypischer Teilung dieses Gewächses kommen bivalente und univalente Chromosomen vor, und letztere führen Längsspaltung zweimal aus, einmal in heterotypischer und einmal in homöotypischer Teilung, was natürlich Gameten mit vermehrter Chromosomenzahl zur Folge hat.

Diese zwei Autoren stimmen aber in der Meinung überein, dass Pflanzen mit vermehrter Chromosomenzahl erst in der F_2-Generation des Bastardes zum Vorschein kommen. Ein ganz abweichendes Resultat bringt aber BREMER's Untersuchung des *Saccharum*-Bastards. Nach ihm tritt vermehrte Chromosomenzahl schon in F_1-Generation auf. Steigerung der Chromosomenzahl dürfte also wenigstens bei diesem Falle in der befruchteten Eizelle stattgefunden haben.

Diese Sachverhältnisse verlocken dazu, weitere Chromosomenforschungen bei verschiedenen Pflanzenbastarden durchzuführen. Vorliegende erste Mitteilung bezieht sich auf den Bastard zwischen *Chrysanthemum marginatum* und *C. lavandulaefolium*. Andere Bastarde unter den verschiedenen *Chrysanthemum*-Arten werden in den nachfolgenden Mitteilungen behandelt werden.

Chrysanthemum ist eine in Bezug auf die Polyploidie der Chromosomen wohl bekannte Gattung. Unter den hier behandelten zwei Arten hat *Chrysanthemum marginatum* als haploide Chromosomenzahl 45, die höchste Anzahl unter den bisher bekannten bei dieser Gattung. Die andere Spezies, *C. lavandulaefolium* zeigt dagegen die niedrigste Anzahl unter der Gattung, nämlich nur 9.[1] Zuerst vermuteten wir bei dem zwischen diesen zwei entstandenen Bastarde etwa ähnliche Verhältnisse, wie sie LJUNGDAHL (1924) seinerzeit beim *Papaver*-Bastade gefunden hat. Zu unsrer grossen Überraschung haben wir aber in der Tat ein ganz anderes Resultat bekommen.

Wie Fig. 1 zeigt, ist der Bastard im Aussehen im grossen und ganzen intermediär im Vergleich mit den elterlichen Pflanzen. Die Kreuzungen geschahen im Herbst 1924 und 1925 nach der Waschmethode, welche COLLINS und MANN (1923) bei ihrer Untersuchung über *Crepis*-Bastarde angewandt haben. Bei der Kreuzung diente *C. marginatum* immer als mütterliche und *C. lavandulaefolium* als väterliche Pflanze. Im ersten Jahre wurden 19 und im nächsten Jahre 215 Köpfchen (mit etwa je 2–5 Einzelblütchen) von *C. marginatum* gekreuzt. Die Fruchtbarkeit ist aber äusserst niedrig; im ganzen haben wir nur etwa ein Dutzend Samen geerntet. Diese wenigen Samen wurden im darauffolgenden Frühling in kleinen Pflanzentöpfen ausgesät. Keimung erfolgte schon nach etwa einer Woche. Während des Sommers wuchsen die Pflänzchen sehr schnell. Doch kam im Herbste nur ein Teil von ihnen zur Blüte. Im nächsten Jahre werden sie alle blühen.[1]

---

[1] Diese Zahlen wurden bei vorliegender Untersuchung von neuem bestätigt.

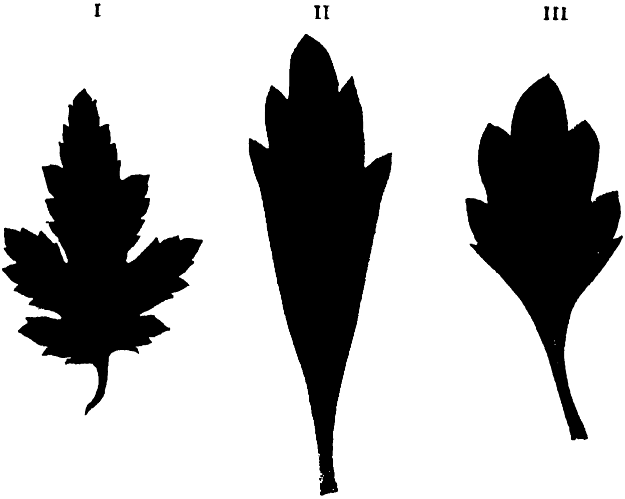


Fig. 1. I. *C. lavandulaefolium*. II. *C. marginatum*. III. *C. marginatum* ♀ × *C. lavandulaefolium* ♂.

Fixierung der Köpfchen dieser Bastardpflanzen ist äusserst schwer. Übliche Fixierungsmittel lieferten uns kein befriedigendes Resultat. Nach wiederholten Bemühungen, speziell für die Fixierung des hier beschreibenden Bastardes, erwies es sich als das beste, Flemmingsche Lösung mit 15 Minuten Vorbehandlung von geschwächtem Gemisch Carnoy's (70% Alkohol 60, Eisessig 30, Chloroform 10) anzuwenden.

Fig. 2 zeigt die Polansicht der heterotypischen Teilung der Pollenmutterzelle. Hier konnten mit Sicherheit 36 Chromosomen gezählt werden. Dieselbe Chromosomenzahl tritt auch bei der homöotypischen Kernplatte (Fig. 3) auf. Die meiotischen Teilungen in den Pollenmutterzellen verlaufen meistens ganz regelmässig.

Ob nun die 36 Chromosomen der heterotypischen Teilung alle bivalent oder einige davon univalent seien, muss durch Untersuchung der somatischen Teilung leicht zu erkennen sein. Deshalb haben wir etwa 20 Stecklinge dieses Gewächses in einem Pflanzentopfe

---

[1] Bis jetzt haben wir nur zwei Individuen cytologisch untersucht. Diese zwei stimmen in ihrer Chromosomen-Garnitur ganz überein.

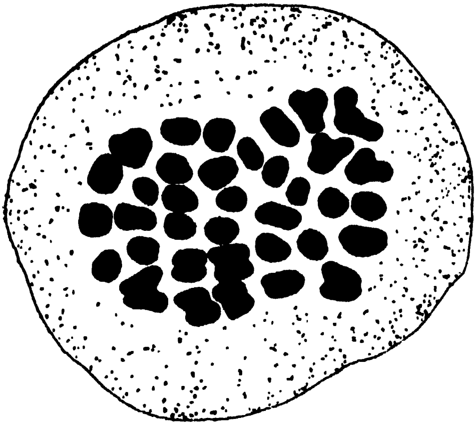


Fig. 2 Heterotypische Teilung der Pollenmutterzelle. ×8150.

gezüchtet. Im Warmhaus aufbewahrt, fingen glücklicherweise schon nach etwa zwei Wochen kräftige Adventivwurzeln an der Basis des Stecklings hervorzutreten an. Diese Würzelchen, mit FLEMMINGscher Lösung (Bonn) fixiert, lieferten uns reichlich Material für somatische Mitosen. Fig. 4 stellt eine Polansicht dieser Teilung dar, wo etwa 72 Chromosomen ohne Schwierigkeit gezählt werden können. Hieraus ergibt sich natürlich der Schluss, dass 36 Chromosomen bei heterotypischer Teilung bivalent sind.

Die obenerwähnten Ergebnisse, dass der Bastard zwischen *C. marginatum* (n=45) und *C. lavandulaefolium* (n=9) bei somatischer Teilung 72, bei meiotischer Teilung 36 Chromosomen aufzuweisen hat, erscheinen auf den ersten Blick ganz unbegreiflich, denn die Summe der haploiden Chromosomenzahl von beiden Eltern macht nur 54 (=45+9) aus.

BREMER's Resultat bei der Untersuchung über *Saccharum*-Bastarde ähnelt gewissermassen unserem Falle. Die haploide Chromosomenzahl beträgt bei einem der Eltern 40 und bei dem anderen 56. Und die des zwischen diesen zweien entstandenen Bastardes beträgt 68. Zur

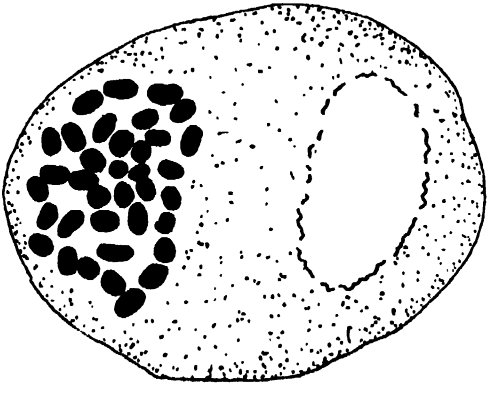


Fig. 3. Homöotypische Teilung der Pollenmutterzelle. ×8150.

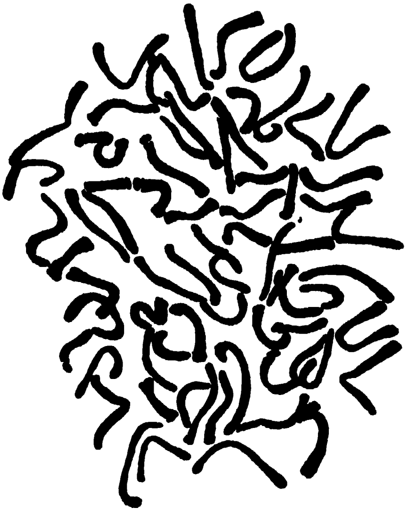


Fig. 4. Somatische Teilung in der Wurzelspitze. × 3400.

Erklärung dieser Erscheinung schreibt er, „During fertilization the *S. officinarum*-chromosomes (n = 40) have undergone longitudinal fission, while those of *S. spontaneum* (n = 56) remained unsplit. This gave a zygote with 136 chromosomes, which by normal division gave rise to an individual with 136 chromosomes in the somatic cells and 68 chromosome pairs in the gonotokonts."

Diese Hypothese genügt aber nicht, die sonderbare Erscheinung bei unserm hier beschriebenen *Chrysanthemum*-Bastarde vollkommen zu erklären, denn bei unserm Falle bekommen wir durch Verdoppelung der väterlichen Chromosomen in der Eizelle nur 63 (= 45 + 9 × 2) Chromosomen; also noch um 9 niedriger als die wirkliche Anzahl. Wir sind darum gezwungen, noch eine Verdoppelung der 9 mütterlichen Chromosomen anzunehmen.

Neuerdings teilten GAINES und AASE (1926) ein neues Beispiel der haploiden Pflanze von *Triticum* mit. Nach diesen Autoren ordnen sich 21 univalente Chromosomen bei der heterotypischen Teilung in der Pollenmutterzelle dieser Pflanze ganz regellos an, ohne dass sich Paarung der eizelnen Chromosomen bildet. Da die Grundzahl der Chromosomen bei dieser Gattung 7 ist (SAKAMURA, 1918), kann die betreffende haploide Pflanze als ein triploides Individiuum betrachtet werden. Das Fehlen der Chromosomenpaarung muss, wie schon die genannten Autoren richtig bemerkt haben, auf die Tatsache zurückgeführt werden, dass die drei Chromosomensätze von diesem Gewächse zu verschieden sind, um Gemini zu bilden.

Es scheint uns also sehr wahrscheinlich, dass auch bei unserem *Chrysanthemum*-Bastarde ähnliche Verhältnisse herrschen. Nämlich einer unter den fünf Chromosomensätzen von *Chrysanthemum marginatum* dürfte etwas anders als die anderen bei der Bastardierung mit

C. lavandulaefolium reagiert haben und Verdoppelung der Chromosomenzahl beim ersten Entwicklungsvorgang der befruchteten Eizelle ausgeführt haben. Also dürften unserer Meinung nach von den 72 Chromosomen des Bastardes 54 $(=36+\overline{9\times 2})$ aus *C. marginatum* und 18 $(=9\times 2)$ aus *C. lavandulaefolium* abgestammt haben.

ZUSAMMENFASSUNG.

1. Bei heterotypischer Teilung der Pollenmutterzelle vom Bastarde zwischen *C. marginatum* (n = 45) und *C. lavandulaefolium* (n = 9) treten 36 Gemini auf.
2. Bei somatischer Teilung in der Wurzelspitze werden 72 Chromosomen gezählt.
3. Steigerung der Chromosomen dürfte dadurch herbeigeführt werden, dass in der Eizelle gleich nach der Befruchtung 9 Chromosomen aus *C. lavandulaefolium* und 9 Chromosomen aus *C. marginatum* sich verdoppeln.
4. Unter den 72 Chromosomen des Bastardes dürften also 54 $(=36+\overline{9\times 2})$ aus *C. marginatum* und 18 $(=9\times 2)$ aus *C. lavandulaefolium* abstammen.

ZITIERTE LITERATUR.

1) Bremer, G. 1923. A cytological investigation on some species and species hybrids within the genus *Saccharum*. Genetica. Bd. 5, s. 97–148, 273–326.

2) Clausen, J. 1926. Genetical and cytological investigations on *Viola tricolor* L. and *V. arvensis* Murr. Hereditas. Bd. 8, s. 1–156.

3) Clausen, R. E. and Goodspeed, T. H. 1925. Interspecific hybridization in *Nicotiana*. II. A tetraploid *glutinosa-tabacum* hybrid, an experimental verification of Winge's hydothesis. Genetics. Vol. 10, s. 278–284.

4) Collins, J. L. and Mann, M. C. 1923. Interspecific hybrids in *Crepis*. II. A preliminary report on the results of hybridizing *Crepis setosa* (L.) Wallr. and with *C. biennis* L. Genetics. Vol. 8, s. 212–232.

5) Digby, L. 1912. The cytology of *Primula Kewensis* and other related *Primula* hybrids. Ann. Bot. Vol. 26, s. 357-388.

6) Gaines, E. F. and Aase, H. C. 1926. A haploid wheat plant. Amer. Jour. Bot. Vol. 13, s. 373–385.

7) Ljungdahl, H. 1924. Über die Herkunft der in Meiosis konjugierenden Chromosomen bei *Papaver* Hybriden. Svensk Bot. Tids. Bd. 18, s. 279–291.

8) Rosenberg, O. 1926. Die semiheterotypische Teilung und ihre Bedeutung für die Entstehung verdoppelter Chromosomenzahlen. Hereditas. Bd. 8, s. 305-338.

9) SAKAMURA, T. 1918. Kurze Mitteilung über die Chromosomenzahlen und die Verwandtschaftsverhältnisse der *Triticum* Arten. Bot. Mag. Tokyo. Vol. 32, s. 151-154.

10) TÄCKHOLM, G. 1922. Zytologische Studien über die Gattung *Rosa*. Acta Horti Bergiani. Bd. 7, s. 97-381.

11) TAHARA, M. 1915. Cytological studies on *Chrysanthemum*. Bot. Mag. Tokyo. Vol. 29, s. 48-50.

12) TAHARA, M. 1915. Cytologische Studien an einigen Kompositen. Journ. Coll. Science. Imp. Univ. Tokyo. Vol. 43, Art. 7, S. 1-51.

13) TISCHLER, G. 1910. Untersuchungen über die Entwicklung des Bananen-Pollens. I. Arch. Zellf. Bd. 5, s. 622-670.

14) TSCHERMAK, E. und BLEIER, H. 1926. Über fruchtbare Aegilops-Weizenbastarde. Ber. Deutsch. Bot. Ges. Bd. 44, s. 110-132.

15) WINGE, Ö. 1917. The chromosomes. Their numbers and general importance. C. R. Trav. Lavor. Carlsberg. Vol. 13, s. 131-275.

16) WINGE, Ö. 1924. Contributions to the knowledge of chromosome numbers in plants. La Cellule. Vol. 35, s. 305-324.

Physiological Studies on the Rhythmical Heart Pulsation in the Oyster, *Ostraea circumpicta* Pils.[1]

By

Shunichi Takatsuki.

(With Two Plates)

There are many investigators who studied the physiology of the hearts of *Mollusca*. Carlson (1905) did the most conclusive work. He studied the physiology of the cardiac nerves in many *Mollusca* (*Mya*, *Tapes*, *Haliotis*, etc.). Mines (1912) studied the physiological action of the inorganic salts on the heart of *Pecten*. Hogben (1925) studied the action of the electrolytes on the hearts of *Pecten* and *Helix*. There are still many others who studied the hearts of *Mollusca* but as far as I am awa[illegible], Tsunoda (1923) is only investigator who studied the action of adrenaline and some of the inorganic salts on the heart of the oyster.

The present investigation was undertaken at the suggestion of Prof. S. Hatai and was carried on at the Asamushi Marine Biological Station.

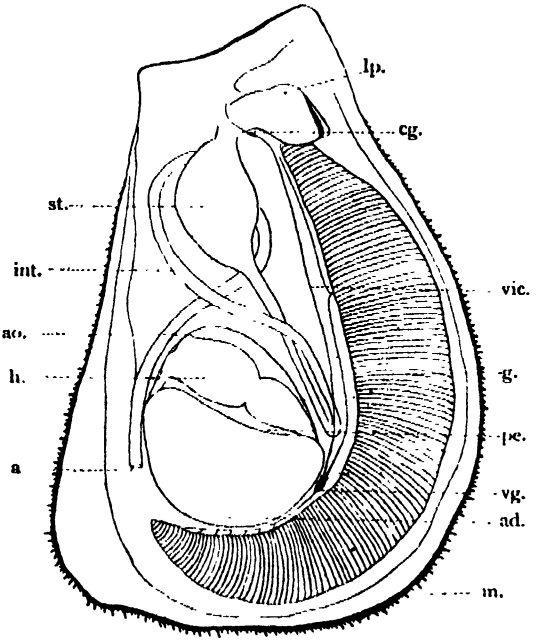
I. THE MATERIAL.

This oyster was collected along the island of Yunoshima near the Asamushi Marine Biological Station. It is a species of considerable size and the total length of the shell often reaches about 17 cm. The heart of the oyster is contained in a pericardium which is situated in front of the adductor muscle of the body. Unlike the majority of *Lamellibranchs*, the heart of this oyster is not traversed by the rectum, and thus it is very favorable material for the study of the physiological characteristics of the pulsatory phenomena. The heart of the oyster consists of a median ventricle and two symmetrically placed auricles of equal size. The wall of the ventricle is composed of muscle fibers which are loosely interlaced. The anterior part of the ventricle is divided into two lateral chambers by a septum which consists of

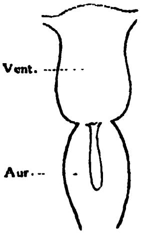
---

[1] A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

muscle fibers. The posterior end of the ventricle gives off two median aorta, one of which runs to the anterior parts of the body while the other goes to the posterior parts. The auricles communicate with the ventricle by a narrow slit on each side. These slits, or apertures, are provided with muscular valves which prevent the reflux of blood from the ventricle. The anterior parts of the auricles communicate with each other within the pericardium. The walls of the auricles are thin, though moderately muscular, and are invested with a brownish coloured epithelium. Along the innermost layers of the auricles are found longitudinal muscle fibers which run parallel to their long axes. (Text Figs. 1 and 2.)



Text Fig. 1. Diagram to show the positions of the heart, alimentary canal, etc. a, anus; ad, adductor muscle; ao, aorta; cg, cerebral ganglion; g, gill; h, heart; int, intestine; lp, labial palp; m, mantle; pe, pericardium; st, stomach; vic, visceral commissure; vg, visceral ganglion.



Text Fig. 2. Diagram to show the heart of the oyster. Vent., ventricle; Aur., Auricle.

II. METHOD OF EXPERIMENT.

The heart was isolated from the body in the following manner: The shell was opened with an oyster-knife and the pericardium was carefully opened. After the posterior end of the ventricle and the anterior ends of the auricles were tied with silk thread, the entire heart was cut off at the closest possible points along the pericardial wall. The heart thus isolated from the body was used to record the rhythmical pulsation on the kymograph by the suspending method.

III. AUTOMATIC RHYTHMICITY OF THE ISOLATED HEART OF THE OYSTER.

Not only the heart, thus isolated completely from the body, pulsates very well in sea water, but even the ventricle or the auricle alone or a piece of the ventricle shows an automatic rhythmicity, as will be shown in Figs. 1, 2, 3, and 4, Pl. 1.

IV. EFFECTS OF VARIOUS KINDS OF PHYSIOLOGICAL SOLUTIONS ON THE ISOLATED HEART OF THE OYSTER.

At first I attempted to determine the effect of several standard physiological solutions on the pulsatory movement of the heart of the oyster.

Experiment 1. Ringer's solution.

In this solution, the heart of the oyster is unable to maintain the normal rhythmical pulsation and becomes gradually weaker and finally stops entirely. The time necessary to abolish the rhythmical pulsation in this solution at a temperature of 14°–15°C. is given in Table 1.

Table 1.

Length of time before heart beat stops; Ringer's solution	0–10 min.	10–20 min.	20–30 min
Number of individuals	13	5	2

It is evident from the table that in the majority of the cases the heart stopped its rhythmical pulsation within the first ten minutes. (Fig. 5, Pl. 1.)

Experiment 2. Ringer-Locke's solution.

This solution, which is considered to be very satisfactory for maintaining the pulsation of the mammalian heart, is unable to sustain the normal rhythm in the oyster, which becomes weaker gradually and finally stops. The time necessary to abolish the rhythmical pulsation in this solution at a temperature of 14°–15°C. is given in Table 2.

TABLE 2.

Length of time before heart beat stops; RINGER-LOCKE's solution	0–10 min.	10–20 min.	20–30 min.
Number of individuals	8	8	4

As is shown in the table, the heart in this solution maintains its pulsation much longer than in RINGER's solution. (Fig. 6, Pl. 1.)

Experiment 3. RINGER-TYRODE's solution.

This solution is highly recommended for studying the intestinal movement of the rabbit. In this solution, however, the heart of the oyster is unable to maintain the normal rhythm, which weakens gradually and finally stops.

The time necessary to abolish the rhythmical pulsation in this solution at a temperature of 13°–14°C. is given in Table 3.

TABLE 3.

Length of time before heart beat stops; RINGER-TYRODE's solution	0–10 min.	10–20 min.	20–30 min.
Number of individuals	11	7	2

As is shown in the table, the heart of the oyster in this solution behaves similarly to that in RINGER's solution. (Fig. 7, Pl. 1.)

In all cases the heart of the oyster stopped its pulsation within half an hour. Although the heart becomes much contracted at the stage when its movement is entirely lost, nevertheless it is not yet dead, because the normal pulsation begins as soon as it is returned to sea water again. It is a well known fact that the cells, unless these are immersed in a solution of which the osmotic pressure is the same as that of the cell contents, will either contract or expand, due to the loss or gain of water, until the osmotic pressure of both solutions is equal. Therefore the difference in the osmotic pressure in the external medium and in the cell will seriously impair the functional activity of living matter.

We found in the above experiments that the solutions thus far tried are unfavorable to the heart of the oyster and, therefore, it is evident that the physiological properties of the heart of the oyster differ from those of the higher animals, due probably to the unfavorableness of the osmotic properties of these solutions on the heart of oyster and not necessarily to the salts contained in the solutions used.

Experiment 4. Saline solution whose osmotic pressure is equal to sea water.

The body fluids of marine invertebrates have the same osmotic pressure as the sea water in which they live, as was shown by BOTTAZZI (1897), FREDERICQ (1901), and others. From this suggestion I employed a saline solution whose osmotic pressure is equal to the sea water in which the oysters live.

In this solution the heart of the oyster can maintain its normal pulsation as long as a week, but after this period its vitality is diminished gradually. The time necessary to abolish the rhythmical pulsation in this solution is given in Table 4. (Fig. 11. Pl. 1.)

TABLE 4.

No. of days before heart beat stops; NaCl solution	0–1	1–5	5–10	Temperature °C.
Number of individuals	2	5	3	15–18

Experiment 5. VAN'T HOFF's solution.

VAN'T HOFF's solution was prepared according to the formula given by him. In this solution the heart of the oyster maintains its normal pulsation as long as 15 days, as is shown in Table 5.

As is shown in Table 5, the duration of the heart pulsation is considerably affected by the temperature of the medium and, indeed, in the lower temperature (7°–14°C.), it survives twice as long as those kept at a higher temperature (23°–25°C.). The present results agree very well with those of TSUNODA (1923), who found the maximum duration of the heart pulsation of the oyster in VAN'T HOFF's solution to be sixteen days. (Fig. 8, Pl. 1.)

TABLE 5.

Duration of pulsation (in days) in VAN'T HOFF's solution	6	7	8	9	10	11	12	13	14	15	16
Number of individuals Temperature 7°-14°C.									1	3	1
Number of individuals Temperature 23°-25°C.	1	3	1								

V. SEA WATER AS A PHYSIOLOGICAL SOLUTION FOR THE HEART OF THE OYSTER.

It is a remarkable fact that the proportion of sodium, potassium, and calcium ions present in sea water is the best proportion to be used for maintaining the beat of the heart, although the degree of concentration must be modified from that of sea water when used for higher terrestrial animals.

According to MAYER (1906), in simple marine animals, such as *Medusae*, *Salpa*, etc, the best solution for pulsation is the sea water itself. This observation of MAYER's holds true for the hearts of *Pecten* (MINES, 1922), *Sepia* (FRY, 1909), *Octopus* (FREDERICQ, 1913), and *Aplysia* (FREDERICQ, 1922), and the present writer also found that sea water is the best physiological solution for the heart of the oyster.

For further reference, I shall give the following data concerning the sea water used in this experiment. The physico-chemical properties of the sea water at Asamushi are as follows.

1) The osmotic pressure of the sea water used in these experiments was determined by the crysoscopic method and its freezing point was found to be about −1.87°C.

2) The chlorine contents of the sea water, determined by titration with standard silver nitrate solution, give about 1.87 gms. in 100 cc.

3) The specific gravity of the sea water, by AKANUMA's hydrometer, is about 1.024–1.023.

4) The hydrogen ion concentration of the sea water, by indicator methods, is about ph. 8.2.

Na.	K.	Ca.	Mg.	Cl.	SO_4.	
1.1634	0.0357	0.0431	0.1373	1.9428	0.2627 gr.	100 cc.

5) According to OKASAKI and KOIZUMI (1926) the sea water at Asamushi has the chemical composition shown in the above table.

Experiment 1.

The heart of the oyster in the sea water maintains its normal rhythmical pulsation for a long period, as is shown in the following table.

TABLE 6.

Duration of pulsation in sea water (average)	Temperature	Number of individuals
15 days	7°–14°C.	5
7 days	23°–25°C.	5

The data given in the table support fully the statements made by MAYER and others that sea water itself is the best medium. The duration of the heart pulsation is affected by the temperature and at a lower temperature (7°–14°C.) the duration of pulsation is twice as long as at a higher temperature (23°–25°C.). The same relation was already found in connection with VAN'T HOFF's solution. (Fig. 1, Pl. 1.)

Experiment 2.

PELSENEER (1906) stated that the number of heart beats of the oyster is twenty times per minute. I found that the number of pulsations of the isolated heart of the oyster varies according to the individual, the temperature, and other factors. The average number found at a temperature of 15°C. was fifteen times per minute. Whether the difference found here is due to the different species or to the isolation of the heart from the body needs further investigation.

Experiment 3.

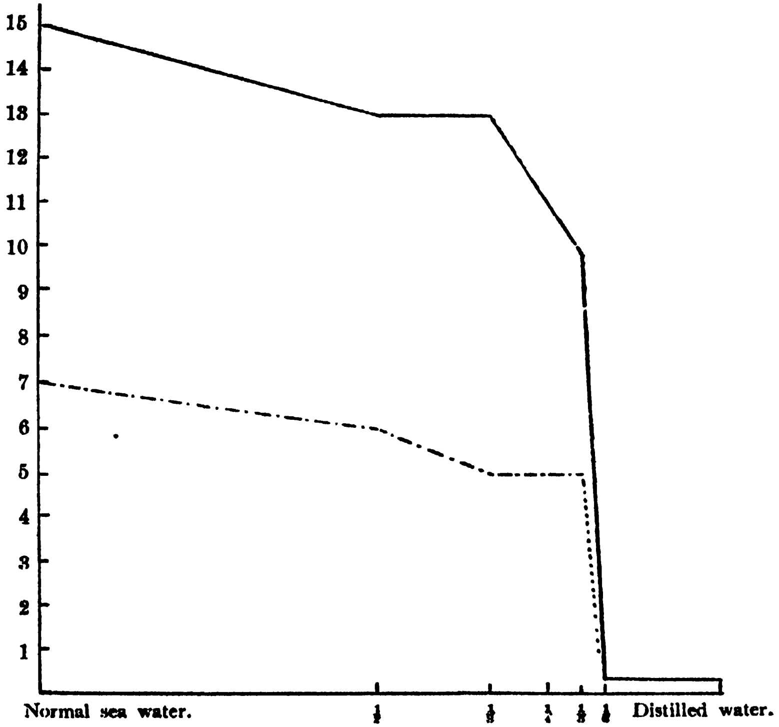
In order to determine accurately how long the oyster heart can survive in sea water of various dilutions, the following experiments were carried out. In general, the greater the dilution of the sea water caused by the addition of distilled water, the shorter was the duration of the life of the heart. The results are shown in the following table.

TABLE 7.

Sea water of various dilutions	Duration of pulsation	Temperature °C.	Number of individuals
sea water	15 days	7.0-15.0	5
	7 days	23.0-25.0	5
1/2 sea water	18 days	9.0-12.5	5
	6 days	23.5-26.0	5
1/3 sea water	13 days	8.0-20.0	5
	5 days	24.0-26.0	5
1/4 sea water	11 days	13.0-18.5	5
	5 days	24.0-26.0	5
1/5 sea water	10 days	15.0-19.0	5
	5 days	24.0-25.5	5
1/6-1/10 sea water	20 min.	7.0-20.0	5

Based on Table 7, Text Fig. 3 was prepared, in which the ordinate indicates the longivity in days and the abscissa the concentration of the sea water. The solid line indicates a temperature of 7°-20°C., the broken line a temperature of 23°-26°C.

The heart in half diluted sea water can survive as long as thirteen days and shows a very slight reduction of activity. Indeed, we often find normal individuals in which the duration of pulsation does not exceed thirteen days even when they are kept in normal sea water. The number of pulsations and the amplitude of contraction are scarcely influenced by such a degree of dilution. In sea water diluted five times, the heart of the oyster still pulsates more than five days, (Temp. 24°-25°C.) but when the sea water is diluted six times, the heart pulsation stops almost as quickly as when treated with distilled water. It is highly interesting and characteristic, also, that the duration of life is diminished only slightly with increased dilution until such a process reaches one-fifth of the normal concentration and that beyond that stage a sudden decline in heart activity is shown.



Text Fig. 8.

VI. PHYSIOLOGICAL ACTIONS OF DISTILLED WATER.

Loeb (1903) found that " if the species of *Gammarus* be transferred suddenly from sea water into distilled water the breathing of the animals ceased in about half an hour, but if the animals are brought directly after the cessation of breathing, back into the sea water they are able to recover. If about ten minutes or longer elapses no recovery takes place." Loeb concludes that " the entrance of the distilled water into the tissue of the animal is responsible for the fatal action." Distilled water, in which ammonia and a trace of copper or other metals was free, was used.

Experiment 1.

If the heart is pulsating in sea water and the sea water is suddenly replaced by distilled water it shows at once reduced contractions of rhythm and this reduction becomes more and more evident, until contraction is completely lost. The heart under such circumstances stops in a systolic condition.

The time necessary to abolish the rhythmical pulsation in distilled water at a temperature of 7°–10°C. is given in Table 8.

TABLE 8.

Length of time before heart beat stops; distilled water	0-5 min.	5-10 min.	10–15 min.	15-20 min.	20-30 min.	20–60 min.
Number of individuals	36	22	17	10	6	2

Table 8 shows clearly that distilled water is injurious to the heart of the oyster and that pulsation stops within a few minutes.

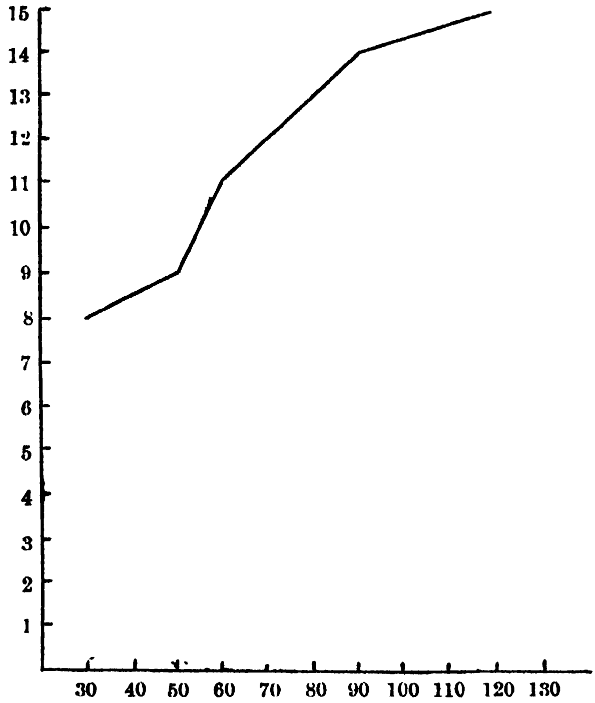
Experiment 2.

If the heart in which pulsation has completely stopped in distilled water is returned to normal sea water within a short period, the pulsation is usually completely restored to the normal state. The time which is necessary to restore its pulsation in sea water after it has been kept in distilled water is shown in Table 9.

TABLE 9.

Duration in distilled water	Time to recover in sea water	Temperature	Number of individuals
30 min.	8 min.	7.5°–8.5°C.	20
40 min.	8.5 min.	8°–8.5°C.	20
50 min.	9 min.	8°–9.5°C.	20
60 min.	11 min	7°–11°C.	20
90 min.	14 min	7.5°–11°C	20
120 min.	15 min.	8°–9.5°C.	20

Table 9. Showing the length of time in distilled water and the length of time necessary to restore the heart beat in sea water.



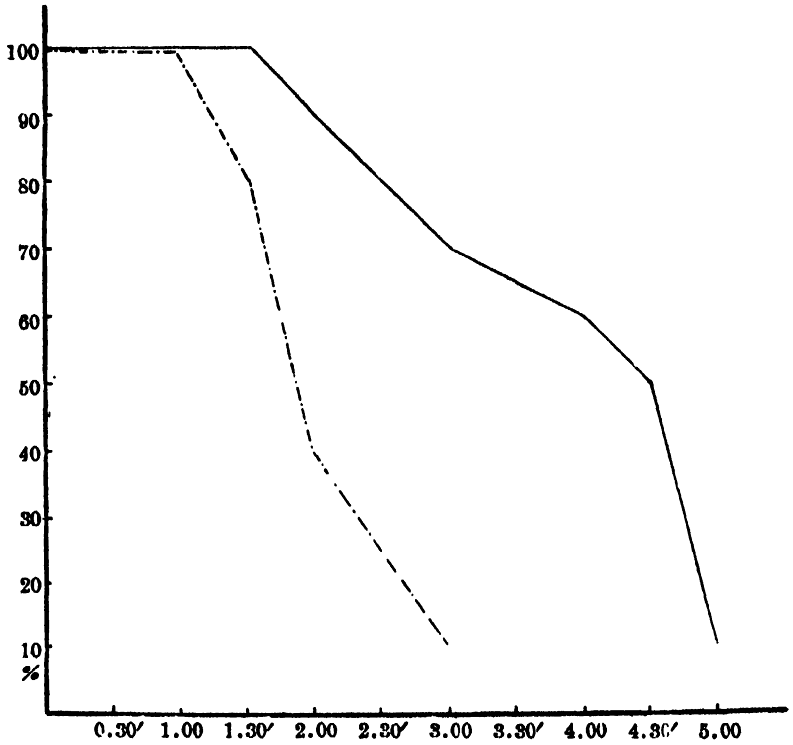
Text Fig. 4. Showing the relation between the time of submergence in distilled water (abscissa) and the time necessary to restore its pulsation in sea water (ordinate).

It is to be noted that the time necessary to restore the pulsation in sea water increases with the prolongation of the duration of submergence in distilled water. The relation just stated appears to be approximately linear. (Fig. 9, Pl. 1.)

Experiment 3.

Experiment 2 shows clearly that the heart of the oyster loses its rhythmic pulsation completely in distilled water but that its rhythm may be restored in sea water if it is replaced within a short period. If, however, the heart is left too long in distilled water, its pulsation cannot be restored in sea water but it dies in a diastolic condition. It was the purpose of the present experiment to determine the maximum length of time for which the heart could remain in distilled water and still be able to recover pulsation when replaced in sea water.

Table 10 shows the results of the experiment. The number of hearts which were recovered in sea water after being kept in distilled water for varying lengths of time are shown in percentages. Each test involves twenty individuals.



Text Fig. 5. The ordinate indicates the % recovered in sea water and the abscissa the length of time, in hours, in distilled water. The whole line indicates a temperature of 8°-13°C. and the broken line a temperature of 21°-26°C.

It is interesting to note that at the lower temperature the heart loses its pulsatory ability in distilled water within ten minutes, but it can survive in that medium for one hour and a half and even after four hours and a half fifty percent of the cases still survived and as many as ten percent survived for as long as five hours. This power

TABLE 10.

No. of hours in distilled water	0.30'	1.00	1.30'	2.00	2.30'	3.00	3.30'	4.00	4.30'	5.00
% of individuals recovered in sea water Temp. 8°–13°C	100	100	100	90	80	70	65	60	50	10
Temp. 21°–26°C.	100	100	80	40	25	10				

to survive is, however, lost much sooner at the higher temperature (21°–26°C.) and only ten percent at this temperature survived after three hours. This shows that the heart of the oyster possessesex traordinary vitality and power to resist unfavourable surroundings.

Experiment 4.

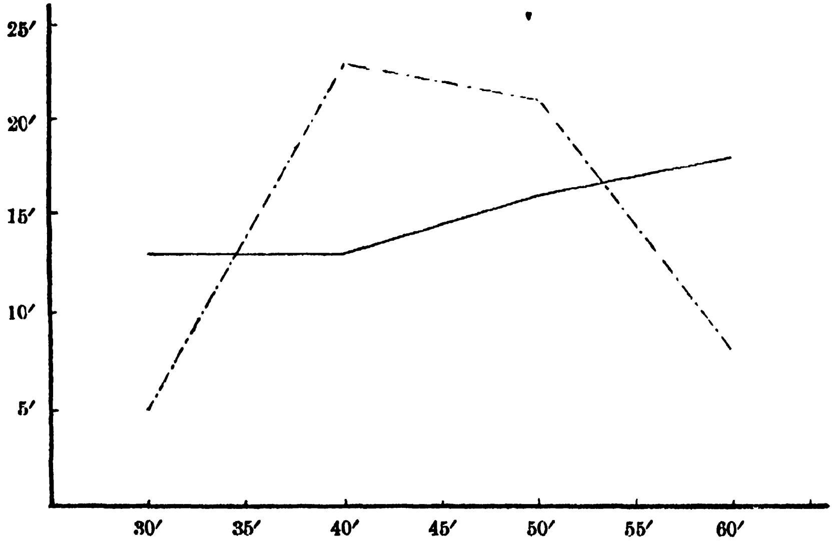
With a view to determine whether or not the heart of the oyster possesses the ability to adapt itself to an injurious medium, I placed it several times in both distilled water and sea water alternately and determined the time necessary to restore its pulsation. It was found that the heart thus treated can prolong pulsation in distilled water and, furthermore, it recovers quicker when replaced in sea water.

Table 11 shows the time necessary to abolish the pulsation in distilled water and then to restore it in sea water.

TABLE 11.

No. of times of placing the heart alternately in distilled water and in sea water	1–3	4	5	6	7	Temperature
Total length of time in minute placing the heart in distilled water	5–20	30	40	50	60	
Time necessary to stop heart beat in distilled water		13	13	16	17	7°–13°C.
Time necessary to restore heart beat in sea water after treatment		5	23	21	8	7°–13°C.

The time required to abolish the pulsating power of the heart in distilled water tends to gradually increase after the treatment of placing the heart in distilled water and in sea water alternately, and the time to restore the pulsation in sea water also tends to increase under the same treatment, with an exception of the last instance, where the



Text Fig. 6. The ordinate indicates the time required to abolish the pulsation of the heart in distilled water and also the time required to restore it in sea water and the abscissa the duration of submergence in distilled water. (Time in minutes) The whole line indicates distilled water, the broken line sea water.

decrease compared with the preceding cases is shown. I, therefore, conclude that the heart of the oyster possesses the power to accustom itself to distilled water if it is placed frequently in both distilled and sea water alternately.

VII. THE ACTION OF THE ELECTROLYTES ON THE HEART OF THE OYSTER.

The relation between the electrolytes present in the external medium and the properties and activities of living cells has been the subject of much investigation ever since Ringer (1882) made his classical investigations on the heart of the frog. From his recent investigations Lillie (1923) concludes that the physiological action of the salts of the medium is upon the protoplasmic surface layer. Whether Lillie's theory can satisfactorily explain the complicated phenomena concerning the properties of the contractile substance of the heart is yet to be studied.

Experiment 1. Single salt.

I have attempted to obtain additional data on the action of each single salt on the rhythmical heart pulsation. The concentration of salt was made equivalent to that in sea water.

1) *NaCl.* LOEB (1903) found that pure sodium chloride is poisonous to the heart tissue, although many other investigators consider that a pure sodium chloride solution is comparatively harmless to living tissue. OVERTON (1904) found that sodium chloride is an essential element for the contractile muscles. HOWELL (1906) stated that sodium ions are essential for the heart rhythm but their function is chiefly to maintain a proper osmotic pressure.

I found that pure sodium chloride solution is comparatively harmless to the heart of the oyster, which maintains pulsation for as long as a week, though its contractility is somewhat weaker when compared with that produced in sea water. (Fig. 11, Pl. 1.)

2) *KCl.* HOWELL (1906) stated that potassium ions seem to be inhibitory in their action upon the heart tissue. HOGBEN (1925) found that the heart of the snail is not insensitive to potassium. TSUNODA (1923) found that potassium ions stimulate the heart of the oyster, but if its content in the medium exceed 0.15% the heart stops in a systolic condition.

My own observation shows also that, in general, the action of potassium is inhibitory in nature and its powerful physiological action may probably be connected with the rapid rate of migration of its ions. A pure potassium chloride solution at first promotes a contractility of the heart, but after a while the pulsation weakens and the heart finally stops in a systolic condition. (Fig. 13, Pl. 2.)

3) *$CaCl_2$.* The necessity of calcium for the heart of vertebrates is well known and LOVATT EVANS (1912) has shown that the same statement applies to that of the snail. The heart of the oyster is powerfully stimulated by a pure calcium chloride solution, but this contractility, as with *KCl*, is soon inhibited and the heart stops in a diastolic condition. (Fig. 10, Pl. 1.)

4) *$MgCl_2$.* TYRODE (1900) stated that the presence of magnesium does not appear to be necessary in an artificial physiological solution. MAYER (1906) and ROGERS (1905) stated that magnesium is an essential

salt for maintaining the pulsation of marine animals. In the heart of the oyster a pure magnesium chloride solution slightly promotes the contractility of the heart, but after a while the pulsating movement becomes less frequent and the heart ultimately stops in a diastolic condition. (Fig. 12, Pl. 2.)

These metalic salts, then, are all essential for the vital activities of living matter, yet a pure solution of each individual salt, with an exception of sodium chloride, can not maintain the rhythmical pulsation of the heart of the oyster for a long period. Even sodium chloride can not be considered entirely free from toxic action, as was already stated above.

Experiment 2. Mixed salts.

In order to maintain the normal rhythmical contraction of the isolated heart most efficiently, a definite proportion of sodium, potassium, and calcium salts is important. I have tried the effect of mixtures of these salts in varying proportions on the pulsation of the heart of the oyster and have obtained the following results:

1) VAN'T HOFF's solution. The rhythmical heart pulsation continues indefinitely, as was already stated above.

2) LOEB (1903) considers that for marine organisms a solution should contain $Na^{\bullet}$, $K^{\bullet}$, and $Ca^{\bullet\bullet}$ ions in a proportion and a concentration equal to sea water. The following proportion and concentration is approximately identical with that of sea water:

100 NaCl + 3,84 $CaCl_2$ + 3,66 KCl. All of 5/8 M.

In this solution the heart of the oyster pulsates normally for as long as a week.

3) LOEB (1906) and MINES (1912) found that the three ions $Na^{\bullet}$, $K^{\bullet}$, $Ca^{\bullet\bullet}$, must be present simultaneousely in the solution used for the prolonged perfusion of the heart, and any one of these three elements can not be replaced by another element. I have removed calcium chloride and replaced it by magnesium chloride.

100 NaCl + 3,84 $MgCl_2$ + 3,66 KCl. All of 5/8 M.

In this solution the heart of the oyster is unable to maintain a rhythmical pulsation for any longer than two or, at most, three hours.

Experiment 3. The effect of the removal of one salt each time (Na, K, Ca, Mg salt) from VAN'T HOFF's solution.

1) Removal of *Na* salt:

5/8 M. (2,2 KCl + 7,8 $MgCl_2$ + 3 $CaCl_2$ + 3,8 $MgSO_4$ + Cane sugar.)

In this solution the heart pulsates abnormally at first and gradually stops within two or three hours. (Fig. 16, Pl. 2.)

2) Removal of *KCl*.

5/8 M. (100 $NaCl$ + 3 $CaCl_2$ + 7,8 $MgCl_2$ + 3,8 $MgSO_4$).

HOGBEN (1925) found that the hearts of both *Helix* and *Pecten* will beat for a considerable length of time in the absence of potassium. In this solution, the heart of the oyster looses its pulsation gradually within a few hours. (Fig. 14, Pl. 2.)

3) Removal of $CaCl_2$.

5/8 M. (100 $NaCl$ + 2,2 KCl + 7,8 $MgCl_2$ + 3,8 $MgSO_4$).

In this solution the rhythmical pulsation weakens rapidly and stops within half an hour. (Fig. 15, Pl. 2.)

4) Removal of $MgCl_2$ and $MgSO_4$.

5/8 M. (100 $NaCl$ + 2,2 KCl + 3 $CaCl_2$).

In this solution the rhythmical pulsation continues longer and normally. The lack of magnesium is, therefore, not so injurious as the lack of each of the three other salts. (Fig. 17, Pl. 2).

These short experiments show the relative importance of the salts used, as well as the action of the individual salts named on the pulsation of the heart of the oyster. However, futher investigations are necessary in order to understand the physiological action of these salts more adequately in relation to the heart movement and therefore I will reserve all these interesting and fundmental investigations for future occassions.

VIII. THE ACTION OF NON-ELECTROLYTE ON THE HEART OF THE OYSTER.

It was found by LOEB (1900) that in a solution of non-electrolyte the isolated center of *Gonionemus* did not exhibit rhythmic contraction. In a medium free from salts the rhythmic contractions are either not initiated or do not continue for any length of time. According to LOEB (1903) "A sugar solution almost isosmotic with sea water is just as poisonous as distilled water to the vital activities of *Gammarus*." In a cane-sugar solution whose concentration is theoretically isotonic with sea water, the heart of the oyster can pulsate as long as two hours, but in a more dilute solution it stops rapidly. The pulsation, when once stopped in this solution, can no longer be recovered in sea water.

IX. THE RELATION OF TEMPERATURE TO PULSATION.

Among the many earlier investigators who studied the effect of temperature on the heart beat, Cyon (1888) accomplished the most conclusive piece of work. He studied the effect of changes of temperature on the number, duration, and strength of the beats of the excised frog heart. There are still many others who studied the effect of temperature on the heart beat. All investigators agree that the number of heart beats increases following a rise of temperature of the medium surrounding the excised heart.

Experiment 1. The effect of temperature on the rate of rhythmic contraction.

The rate of rhythmic contraction of the heart of the oyster was continuously observed during the entire course of each experiment; that is, either a gradual decrease down at 0°C. or a gradual increase till the heart movement was stopped altogether. In the oyster the pulsation of the heart is entirely abolished when it is exposed at 0°C., and at 5°C. it begins to pulsate slowly and irregularly though the amplitude of contraction is comparatively large. At 7°C. the pulsation becomes normal and a further gradual rise of temperature gradually accelerates pulsation, which in turn is followed by a slow decrease of the contraction height. At 35°C. the number of pulsations per minute reaches the maximum. At 45°C. it is permanently abolished. (Figs. 18 and 19, Pl. 2.)

The relation between the temperature and number of pulsations can be expressed by the equation $F=kT^i$ in which k and i are constant, F, the number of pulsations per minute, T, temperature in °C.

The constant was calculated according to a convenient method devised by Nomura (1926).

$$F=0.30\times T^{1.29}.$$

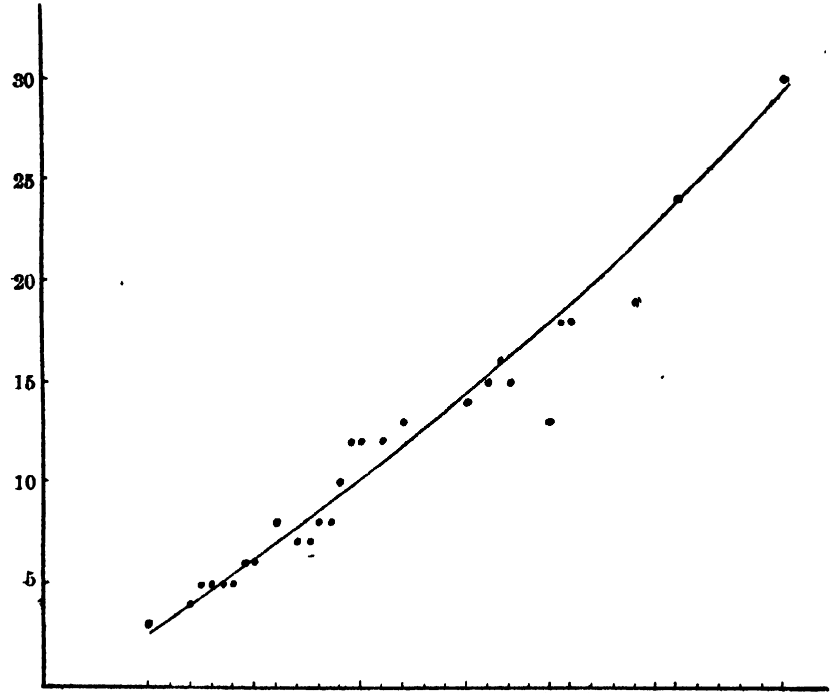
Table 12 shows the relation between the temperature and the number of pulsations.

TABLE 12.

Temperature °C.	No. of pulsations per minute	No. of Calculated pulsations $F=0.30\times T^{1.29}$	Number of individuals
0	0	0	20
5	3	2.4	20
7	4	3.7	5
7.5	5	4.0	16
8	5	4.4	92
8.5	5	4.7	44
9	5	5.1	42
9.5	6	5.5	16
10	6	5.9	27
11	8	6.6	3
12	7	7.4	21
12.5	7	7.8	42
13	8	8.2	34
13.5	8	8.4	13
14	10	9.0	37
14.5	12	9.4	18
15	12	9.8	23
16	12	10.7	24
17	13	11.6	31
20	14	14.3	25
21	15	15.2	13
21.5	16	15.7	7
22	15	16.1	5
24	13	18.0	29
24.5	18	18.3	10
25	18	19.0	3
28	19	22.0	20
30	24	24.1	20
35	30	29.4	20
40	14		20
45	0		20

Based on Table 12, Text Fig. 7 was prepared in which the ordinate indicates the number of pulsations and the abscissa the temperature of the medium.

The points denote the relations between the number of pulsations per minute and the temperature obtained from actual observations and the line denotes the relation expressed by the equations ($F=0.30 \times T^{1.20}$).



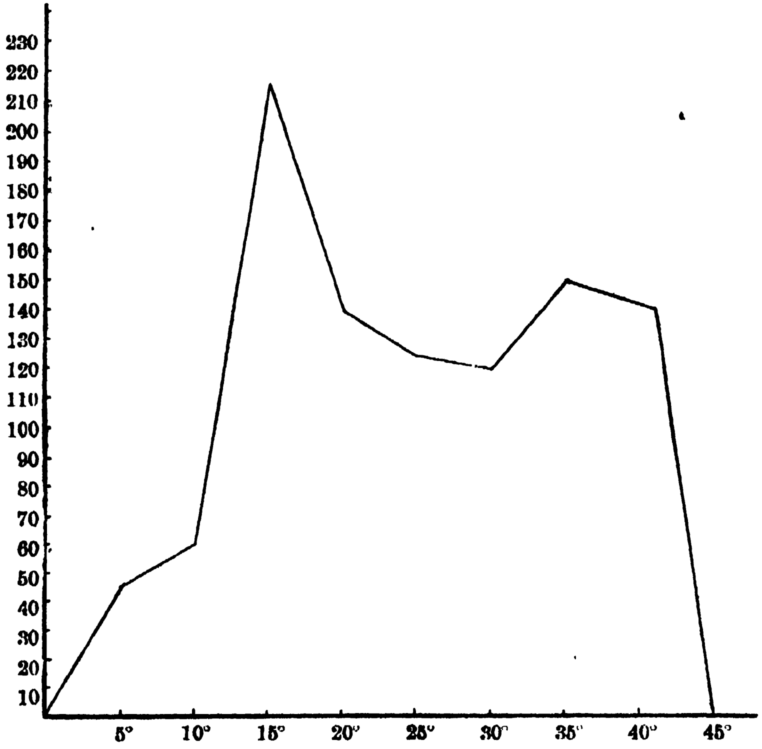
Text Fig. 7.

Experiment 2. Temperature coefficient of the pulsation.

ROGERS (1913) has shown that in the *Fundulus* embryo the temperature coefficient of the rate of rhythmical pulsation of the heart is of the order of magnitude of a chemical reaction. MOORE (1918) stated also that "the temperature coefficient of the rate of heart beat of *Fundulus* embryo resembles closely to an emzyme action and the values of temperature coefficient (Q_{10}) are shown to be higher at

TABLE 13.

Temperature °C.	Q_{10}	Temperature °C.	Q_{10}
5....15	4.0	14....24	1.3
7....17	3.3	14.5..24.5	1.5
10....20	2.3	15....25	1.5
11....21	1.9	20....30	1.5
12....22	2.1	25....35	1.7



Text Fig. 8. The ordinate indicates the works done by the heart of the oyster (number of pulsations per minute × amplitude of contraction) and the abscissa the temperature of the medium.

temperatures near zero and to decrease, as is the case with enzyme, when the temperature is raised." There are still many others who studied the temperature coefficient of pulsation.

The temperature coefficient of the rate of the heart beat of the oyster was calculated for 10°C. intervals and the results are given in Table 13.

Experiment 3.

The work done by the heart of the oyster (number of pulsations per minute × amplitude of contraction) may be shown in the curve. As will be seen in Text Fig. 8 at a temperature of 15°C. the heart performs the maximum work, which is followed by a rapid decrease with an increasing temperature up to about 30°C. At 35°C. it again rises and then sinks to minimum at 45°C.

X. SUMMARY.

1. The gross anatomy of the heart, as well as the method of its isolation from the body, is given.

2. Physiological solutions of RINGER'S, RINGER-LOCKE'S, and RINGER-TYRODE'S are not suitable for the heart of the oyster. Sea water itself and VAN'T HOFF'S solution are very excellent media for the heart of the oyster.

3. The isolated heart of the oyster can maintain pulsation in either sea water or in VAN'T HOFF'S solution as long as sixteen days. The number of pulsations of the isolated heart vary according to the temperature, but at 15°C. the average number is 15 per minute.

4. The heart of the oyster is able to maintain a pulsation for 10 days in sea water which was diluted five times with distilled water.

5. Distilled water is poisonous to the heart of the oyster and soon abolishes pulsation completely, but of the hearts which were placed in distilled water for a period of two to four hours, fifty percent recovered normal pulsation when replaced in sea water.

6. NaCl, KCl, and $CaCl_2$ are the salts essential for maintaining the rhythmical pulsation of the heart. Calcium salt can not be replaced with other salts. Magnesium salt is also essential for maintaining the pulsation of the heart of the oyster.

7. The pulsation of the heart of the oyster is completely abolished at 0°C but pulsation begins at 5°–7°C., and the number of pulsations is a logarithmic function of temperature. The temperature coefficient for 10°C. is about 2.1.

Before leaving the subject, I desire to express my sincere thanks to Prof. S. HATAI, Director of the Asamushi Marine Biological Station, for his kind guidance and encouragement. I am also indebted to Prof. E. NOMURA for his kindness in giving me valuable criticism.

LITERATURE.

1) BAYLISS, W. M. 1920. Principles of general physiology. London.
2) BENEDICT, S. R. 1905. The influence of salts and non-electrolytes upon the heart. Amer. Journ. Physiol., Vol. 22
3) CARLSON, A. J. 1906. Vergleichende physiologie der Herznerven und der Herzganglien bei der Wirbellosen. Ergeb. Physiol., Vol. 8.
4) DAHMEN, P. 1923. Anatomie von *Ostrea chilensis* PHIL. Jenaische Zeitschr. f. Naturwissensch., Vol. 61.
5) HÖBER, R. 1922. Physicalische Chemie der Zelle und Gewebe. Leipzig.
6) HOGBEN, L. T. 1925. The action of electrolytes on invertebrate muscle. Quart. Journ. Exper. Physiol., Vol. 15.
7) HOWELL, W. H. 1906. The cause of heart beat. Journ. Amer. Medic. Assoc., Vol 46.
8) LILLIE, R. S. 1923. Protoplasmic action and nervous action. Univ. Chicago Press.
9) LOEB, J. 1903. On the relative toxicity of distilled water, sugar solution and solutions of the various constituents of sea water for marine animals. Univ. California Public., Physiol., Vol. 1.
10) LOEB, J. 1906. Dynamics of living matter. London
11) MAYER, A. G. 1906. Rhythmical pulsation in *Scyphomedusae*. Carnegie Int. Public.
12) MINES, G. R. 1912. On the functional analysis by the action of electrolytes. Journ. Physiol., Vol. 43.
13) MOORE, M. M. 1918. Evidence for enzymatic basis of heart-beat. Amer. Journ. Physiol., Vol. 45.
14) NOMURA, E. 1926. An Application of $a=kb^x$ in Expressing the Growth Relation in the Freshwater Bivalve, *Sphaerium heterodon* PILS. Science Reports Tohoku Imp. Univ., Biology, Vol. 2.
15) OKAZAKI, K. and KOIZUMI, T. 1926. Über die Leibeshöhlenflüssigkeit von Holothurien, *Caudina chilensis* (J. MÜLLER) Science Reports Tohoku Imp. Univ., Biology, Vol. 2
16) PELSNEER, P. 1906. A treatise on Zoology, Part V. *Mollusca*. London.
17) ROGERS, C. R. 1906. The effect of various salts upon the survival of the invertebrate heart. Journ. Exper. Zool., Vol. 2.
18) TSUNODA, H. 1923. The abstract of annual meeting of the Physiological Society of Japan.
19) WINTERSTEIN, H. 1925. Handbuch der vergleichende Physiologie, Bd. I. I. Hälfte.

EXPLANATION OF PLATES.

PLATE 1.

(The time marked per minute.)

Fig. 1. Showing the rhythmical pulsation of the isolated heart of the oyster in sea water. Temperature 10°C.

Fig. 2. Showing the rhythmical pulsation of the ventricle alone in sea water. Temperature 10°C.

Fig. 3. Showing the rhythmical pulsation of the auricle alone in sea water. Temperature 10°C.

Fig. 4. Showing the rhythmical pulsation of a piece of the ventricle in sea water. Temperature 10°C.

Fig. 5. Showing the rhythmical pulsation to cessation in RINGER's solution and restoration by normal sea water. Temperature 10°C.

Fig. 6. Showing the rhythmical pulsation to cessation in RINGER-TYRODE's solution and restoration by normal sea water. Temperature 10°C.

Fig. 7. Showing the rhythmical pulsation to cessation in RINGER-LOCKE's solution and restoration by normal sea water. Temperature 10°C.

Fig. 8. Showing the rhythmical pulsation in VAN'T HOFF's solution. Temperature 10°C.

Fig. 9. Showing the rhythmical pulsation to cessation in distilled water and restoration by normal sea water. Temperature 11°C.

Fig. 10. Showing the rhythmical pulsation to cessation in pure calcium chloride solution and restoration by normal sea water. Temperature 10°C.

Fig. 11. Showing the rhythmical pulsation in pure sodium chloride solution. Temperature 11°C.

PLATE 2.

Fig. 12. Showing the rhythmical pulsation to cessation in pure magnesium chloride solution. Temperature 10°C.

Fig. 13. Showing the rhythmical pulsation to cessation in pure potassium chloride solution. Temperature 10°C.

Fig. 14. Showing the effect of the removal of potassium from VAN'T HOFF's solution. Temperature 12°C.

Fig. 15. Showing the effect of the removal of calcium from VAN'T HOFF's solution. Temperature 12°C.

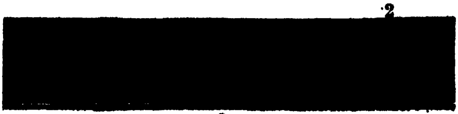
Fig. 16. Showing the effect of the removal of sodium from VAN'T HOFF's solution. Temperature 12°C.

Fig. 17. Showing the effect of the removal of magnesium from VAN'T HOFF's solution. Temperature 12°C.

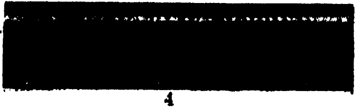
Figs. 18 and 19. Showing the relation between temperature and pulsation.

1

2



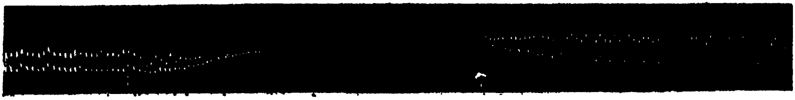
3



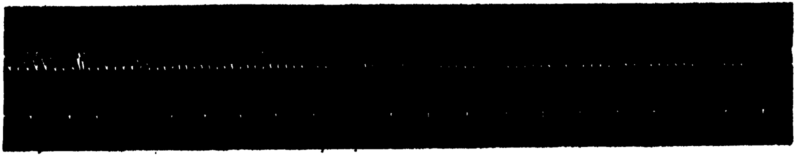
4

5

6

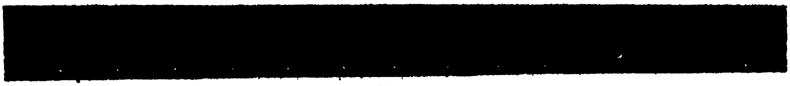


7

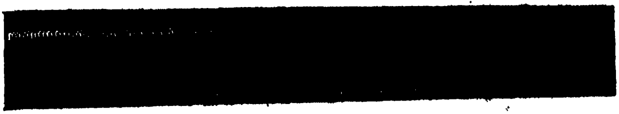


8

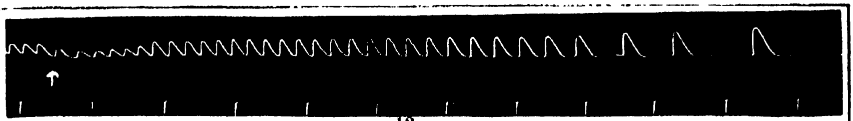
9



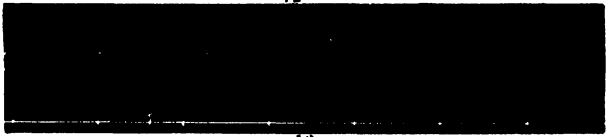
10



11



12



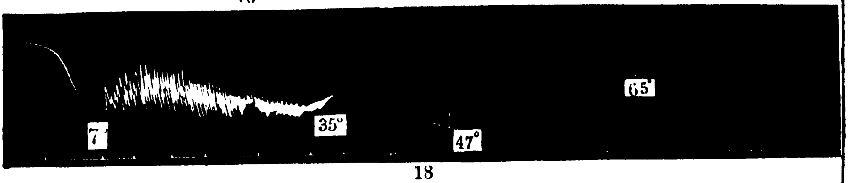
13

14

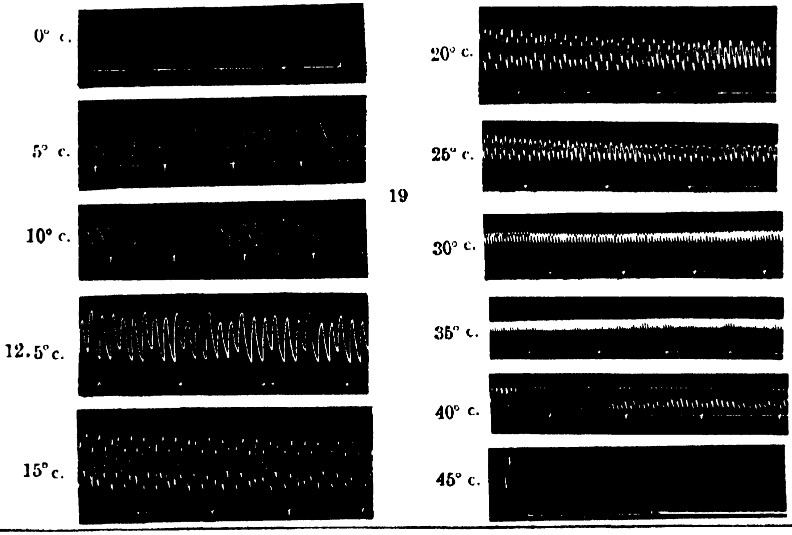
15

16

17



18



19

Contribution to the Research on the Respiration of Fishes.

I. On the Hydrogen Ion Concentration and the CO_2-gas Content and Capacity of Fish Blood.*

By

SEIJI KOKUBO.

We have already a considerable number of works concerning the hydrogen ion concentration of blood, which have been suddenly added during the last two decades. The introduction by CULLEN (1922) of an unique method of colorimetric determination of the pH value of blood much accelerated the study of this relation. These are, however, almost confined to normal and pathological conditions of man and several common mammals, while the fish has scarcely been investigated in this regard, and the invertebrates, such as insects, and etc., have only been touched upon.

In the air-breathing land animals the blood is in contact with the atmosphere while it is passing through the capillaries of the lungs. In the water inhabitants, such as the fish, the blood in the capillaries of the gills is brought, on the contrary, into direct contact with the water. As the atmosphere, which is the respiration medium of land animals, has a very constant character in its composition, the tension of gases do not particularly influence the blood of animals, unless the animals go to an extraordinary high altitude or unless the respiration is done in an abnormal manner, as in the case of pathological or anesthetized conditions. Indeed, the blood of land animals has but an indirect relation upon the respiration medium concerning the hydrogen ions, giving off the CO_2-gas of the blood into the air and thus decreasing the hydrogen ion concentration of the blood, or, inversely, diffusing the CO_2-gas of the air into the blood and thus increasing the hydrogen ion concentration of the latter. With regard to the respiration medium of hydrobios, i. e., water, however, not only the pressure relation of O_2 and CO_2 differs from that of the atmosphere, but also this relation varies according to biological and physical causes, and, in addition, the blood of water inhabitants contacts directly the hydroxyl or hydrogen ion of the water and the reaction of the latter has a direct relation upon the blood reaction. Accordingly, the regulation of the reaction, that is to say, the controlling of the acid and base equilibrium, must be an indispensable faculty of hydrobios.

---

* A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

POWERS (1922) investigated the fish blood from this point of view and stated that the acid-base equilibrium is somewhat rapidly changed in some fish for the purpose of accomodation to the environment. The method he employed was, however, MARIOTT's colorimetrical method which considers the pH value as the relative alkaline reserve of the blood and hence the actual alkaline reserve, i. e., the content of the CO_2-gas of the blood, was not estimated in his case.

The present investigation was undertaken to throw some light upon the relation of the hydrogen ion concentration to the CO_2-gas content of fish blood, and to point out the difference which may exist among several species of fishes and between them and the mammalian. I have chosen as material fish from fresh and from sea water. As is familiar to the student of haemology, the fish blood differs a good deal from the mammalian, at least in regard to the morphology of the corpuscles; some further differences may be expected as regards the concentration of the hydrogen ion when it is made clear.

As experimentally well known, the acidosis of land animals can be brought about by an acid containing diet or by the injection of acid into the circulatory system. In water breathers acidosis is quite easy to cause by simply changing the hydrogen ion concentration of the water used for respiration. It follows that the hydrogen ion concentration and the CO_2-gas content of fish blood can be altered by the environmental condition, hence I have endeavoured to estimate the CO_2-content and the CO_2-capacity together with the pH value.

The study of the hydrogen ion concentration was carried out by the colorimetric method, and the CO_2-gas volume was determined exclusively by the VAN SLYKE blood gas apparatus.

The material employed consisted of two species of fresh water fish, six species of sea fish, a species of Echinodermata (*Caudina*) and a Mollusca (*Arca*), as the following table shows:

1. *Mustelus manazo* (Hosizame)

2. *Cyprinus carpio* (Carp)
3. *Salmo iridius* (Rainbow trout)
4. *Leuciscus hakuensis* (Ugui)
5. *Seriola aureovittata* (Buri)
6. *Pseudomonacanthus modestus* (Umadurahagi)
7. *Lateolabrax Japonicus* (Suzuki)
8. *Pagrosomus major* (Tai)
9. *Caudina chilensis* (Sironamako)
10. *Arca inflata* (Akagai)

Here I express my heartiest thanks to Prof. S. HATAI for his kindness given in the course of the present investigation, especially in the preparation of the manuscript.

I. METHOD.

1. Blood collection.

a. Method of blood collection. As the fish heart is of very small bulk, bleeding is not easy in contrast to the mammalian heart, from which the blood can be obtained simply by a syringe after incising the thoracic cavity. While the thoracic muscles of fish are very thick and strong the heart and the blood vessels are exceedingly delicate, and, furthermore, the heart is situated not only deep, but the pulsation is feeble and, in addition, its position is highly variable in different species. The operation is therefore by no means easy, and there is necessity of some exercise of skill in withdrawing the blood without touching the air, especially from floundering fish. I always used the heart puncture in collecting the blood. As to the precise method of puncturing, it will be mentioned under each species which is dealt with in the following.

In *Cyprinus*, with which the description is commenced, the heart is situated on the line connecting the posterior end of the basal borders of the pectoral fins on both sides. Accordingly, the blood may be obtained by inserting the syringe needle into the middle point of this line. In *Salmo* the insertion must be made in front of this line, as the heart is found a little more anteriorly. *Leuciscus* has the heart in a little anterior part of the line connecting the anterior end of the basal line of the pectral fins, so the syringe needle is inserted in the middle point of the line which connects the posterior end of the operculum. *Seriola* is operated upon the line connecting the bases of the subopercula on both sides, because the heart is a little anterior to the line connecting the posterior end of both the opercula. In the laterally compressed *Pseudomonacanthus*, the syringe needle is inserted from the lateral side at a height immediately anterior to the clavicle bones, between which the heart is situated. In this fish the blood drawing is not difficult owing to the moderate size of its heart. In *Lateolabrax* and *Pagrosomus* the operation is made in front of the line connecting the posterior margins of the preopercula on both sides and on the level of the base of the pectral fins and just anterior to the preopercula, where the heart is most conveniently punctured.

In *Mustelus*, whose sinus venosus is situated dorsally and posteriorly to the ventricle, the needle is operated obliquely dorsally and posteriorly from the ventral median zone between both the pectoral fins, so that it passes through the ventricle from the ventral surface and pierces the sinus venosus, from which the blood is withdrawn.

In the case of *Caudina* the bleeding can easily be accomplished protecting the blood from the air either by incising the body wall under paraffin oil or by inserting the syringe needle into the body cavity of the animal. As to *Arca*, the method is quite different. By removing the shell from the animal, the heart is exposed *in vivo* and the blood is collected from the heart into the syringe in the ordinary way.

b. Blood collecting apparatus. The apparatus, with which the blood was taken out, was a syringe and a spray bulb. The syringe consists of a needle and a 1 cc. pipette graduated to hundredths, or sometimes a 2 cc. pipette graduated to fiftieths of 1 cc. The spray bulb was furnished simply with a rubber tube and the pippette was applied on the distal end of the latter when the apparatus was employed for blood suction. At the time of use, the terminal part of the pippete was filled with paraffin oil to prevent the contact of the blood with the air. The blood then contained in the pipette was discharged into a centrifuge tube containing the saline indicator solution, which was covered with a thin layer of paraffin oil.

2. Colorimetry.

There have hitherto been advanced three different methods for the colorimetric estimation of the pH value of blood. In 1915, ROWNTREE and MARIOT formulated for the first time a colorimetric method which awakened the lively interest of heamologists. This method consists in a dialysate of the blood which is obtained by using a semipermeable membrane and is then turned into the proper colour by the addition of an appropriate indicator. In his recent paper, CULLEN (1922) gives a very practical method, which is very simple and consists essentially in the use of a nonbuffer saline solution having a definite pH value and already containing an indicator. The pH value is determined by comparing the serum diluted with this solution with the colour standard. This method, with certain modifications made by MYER and HAWKINS, is now most extensively used. In their latest publication, HOLLO and WEISS (1924) give a new method in which the plasma, diluted 14 fold with the buffer saline solution containing neutral red, was used as the colour standard. On the other hand, 0.5 cc. of phosphate mixture is added to 2.5 cc. of the 10 fold diluted plasma and is titrated by dropping n/100 HCl solution until the colour of the solution becomes just the same as the colour standard. The pH of the original plasma can be obtained from the volume of HCl used in titration.

Of the three above mentioned procedures, the method by HOLLO and WEISS is not yet confirmed by others, but Cullen's method was preferred to ROWNTREE's and is now affirmed and recommended by several authors. In the present work I employed this method with some modifications concerning minor points, which are given in the following.

a. Saline solution. The saline solution used to dilute the plasma was prepared by re-distilled water free from CO_2. To drive out the CO_2 from the re-distilled water I boiled it in a pure pyrex flask and introduced the CO_2-free air when it cooled. Then NaCl and the indicator (0.02% phenol red) was added and the pH was adjusted to 7.40. As the reaction of this solution is very delicate, it always shows a tendency to become acid, so that it was extremely difficult to keep the correct pH unchanged for a long time. To overcome this difficulty I used an ordinary pyrex flask provided with a rubber stopper through which two glass tubes were passed. One of these glass tubes reached only to the inner surface of the cork, while the other one was so long as to reach almost to the bottom of the bottle. The solution which was prepared flowed out from the shorter glass tube when the flask was turned upside down; the air free from CO_2 was introduced into the bottle through the longer glass tube. Even in this case a slight change of the pH of the saline solution could not be avoided, but a change less than pH 0.1 did not affect the result.

For the isotonic concentration of the saline solution, 0.8% was preferred for fish blood, while 3.5% was used for the blood of *Mustelus*, *Caudina*, and *Arca*.

b. Colorimetric standard. For the colorimetric standard of the pH of blood, SORENSEN's phosphate mixtures are commonly made from an m/15 solution of secondary sodium phosphate (Na_2HPO_4) and primary potassium phosphate (KH_2PO_4). In the present investigation the primary and secondary sodium phosphate was made from Merk's NaOH and phosphoric acid in the following way:

10 cc. of phosphoric acid was diluted with 100 cc. of distilled water and titrated with 1 normal NaOH solution, using phenolphthalein as the indicator. The titration was finished when a distinct rose colour appeared. If in this case, 10 cc. of diluted phosphoric acid neutralised 20 cc. of 1 normal NaOH solution, it could be taken as a correctly 1 mol. solution. The m/15 primary and secondary phosphate could be obtained from this 1 mol. solution by the following way.

1) m/15 Prim. Sodium Phosphate....10 cc. of 1 mol. phosphoric acid + 10 cc. of 1 normal NaOH solution + 130 cc. of distilled water.
2) m/15 sec. Sodium Phosphate....10 cc. of 1 mol. phosphoric acid + 20 cc. of 1 normal NaOH solution + 130 cc. of distilled water.

The proportion of these two solutions, which are thus mixed, is the same as those ordinarily used by mixing a solution of crystalline phosphate salts. In my case nine different standards were employed covering the pH range from 87.0 to 8.8, the standards differing by 0.1 of pH, while CULLEN used in his investigation colour standards differing by 0.05 of pH. Each colour standard contained 5 cc. of phosphate mixture with 0.25 cc. of 0.02% phenol red solution in a pyrex test tube 14 mm. in diameter.

c. Dilution. According to the results obtained by CULLEN, horse serum shows a continued increase in alkalinity when it is diluted with

0.9% NaCl solution up to a dilution of 15 to 20 fold and beyond a 20 fold dilution the change of alkalinity becomes so small as to be practically negligible. This is the reason why he chose the 20 fold dilution as optimal. Whilst in my experience with Cavia a similar result, such as CULLEN's was reached, but the fish in this respect was quite different and the pH never reached any fixed limit associated with the dilution, thus it seemed advisable to choose as many different dilutions as possible. I have limited my observation to the following dilutions: 6, 11, 21, 51, and 101 fold with saline solution. As 2 cc. of blood was collected from a fish at one time in most cases, several grades of dilution were easily prepared.

d. Correction. In the warm blooded animals, like the Mammalia, the blood reaction ought to be tested under body temperature, or if tested under room temperature the result must be converted into that of body temperature. In fish, however, the body temperature is controlled by the water temperature in which they live, and ranges, in general, between 0°–28°C. In my investigation the saline solution and the colour standard were kept for long time in the same room where the temperature variation war very slight, and as the addition of blood hardly alters the temperature of saline solution, the colorimetric reading was directly made without further correction for temperature variation in all cases.

3. Estimation of the CO_2-gas content and capacity.

For the estimation of the CO_2-gas content and capacity VAN SLYKE's blood gas apparatus was employed, in every case either whole blood or serum was used. In the case of whole blood, 10% lactic acid, and in the case of serum 5% solution of H_2SO_4 was used.

To determine the CO_2-capacity, 2 cc. of oxalated plasma was placed in the tonometer of about 350 cc. capacity, introducing the alveolar air into the tonometer. The equilibrium with the alveolar air is brought about by the tonometer, which was shaken for one and a half minutes in such a way that the blood was evenly distributed along the wall of the tonometer and the shaking continued for half a minute more.

The carbon dioxide content and capacity of the blood and the coelomic fluid of marine forms was investigated by COLLIP (1922). His estimation, however, was accomplished with blood shed from severed caudal vessels, in the case of fish, and hence the blood was naturally exposed to the atmosphere. Therefore the value which he calls the carbon dioxide content can not be absolute, but only a relative alkali reserve of the blood, because the blood will liberate the carbon dioxide if it was equilibrated with the atmospheric air. Consequently, the figure which he shows as the carbon dioxide content must indicate a somewhat smaller value than in the natural state. In my investigation, however, the blood used was drawn directly from the heart by a puncture and as it was never exposed to the air, the results show the carbon dioxide content in the true sense in all instances.

II. EXPERIMENTS AND RESULTS.

EXPERIMENT I. Hosizame (*Mustelus manazo*).

A. Case history.

Hosizame is a kind of common shark, an yearly frequenter in Aomori Bay during July. The fish which I employed in the experiment was caught on the beginning of July and was kept until the experiment. It was 42 to 79 cm. in length and 181 to 1385 gms. in weight. Though the blood can be withdrawn by a heart puncture, as already described, a quantity of blood may also be collected by inserting the syringe needle into the sinus venosus when the abdomen is cut open. The blood from one individual was sufficient to determine the pH of varying dilutions and the CO_2-gas content of the whole blood.

B. Results.

1. *Hydrogen Ion Concentration.* Alkalinity of the blood of this fish was very high in comparison with that of the other fishes. It showed a pH value of 7.65 in 6 fold dilution ; in 11 to 21 fold dilution, however, it becomes 7.72, showing a slight increase of alkalinity. By further dilution the alkalinity again diminished, indicating pH 7.69 in 51 fold dilution and 7.63 in 101 fold dilution.

2. *CO_2-gas content.* According to the eight determinations recorded in the Table I, the CO_2-gas content of this blood showed 7.8 to 13.2 vol. % with the average value of 9.1 vol. %. This content is a little smaller than that of the Umazurahagi (*Pseudomonacanthus*

modestus). Between the pH and the CO_2-gas content some parallelism is seen. For instance, the Table I showed the CO_2-gas content of 13.2 vol. % in No. 8 and 9.3 vol. % in No. 4 and in correspondence with this the pH values in 11 fold dilution were also as high as 7.80 and 7.77 respectively.

TABLE I.

Case No.	pH Degree of dilution					CO_2-gas Content vol. %	Dimensions of fishes			Time (1925)		
	6 fold	11 fold	21 fold	51 fold	101 fold		Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	—	7.75	7.73	7.70	7.65	7.8	48.0	3.8	269	VII	27	9.00a.m.
2	—	7.73	7.73	7.70	7.64	7.8	47.0	3.9	277	VII	27	9.30a.m.
3	7.65	7.70	7.68	7.62	7.58	7.8	42.0	3.0	195	VII	27	10.00a.m.
4	—	7.77	7.77	7.74	7.69	9.3	44.7	3.5	225	VII	27	10.25a.m.
5	—	7.65	7.66	7.65	7.60	8.8	57.0	4.0	463	VII	27	10.50a.m.
6	—	7.74	7.74	7.72	7.54	9.0	47.0	3.2	181	VII	27	11.25a.m.
7	—	7.64	7.65	7.64	7.60	8.8	57.8	4.7	479	VII	27	11.45a.m.
8	7.65	7.80	7.80	7.75	7.73	13.2	79.0	5.8	1385	VII	27	12.10p.m.
Mean	7.65	7.72	7.72	7.69	7.63	9.1	—	—	—	—	—	—

The pH and the CO_2-gas content of the blood of *Mustelus*.

EXPERIMENT II. Carp (*Cyprinus crapio*).

A. Case history.

The carps used for this experiment were cultured in the culture pond of the University of Sapporo and were kept thereafter in another small pond ever since 1923. Before the experiment the fishes were put about one week in an aquarium of the laboratory. The reaction of the pond water was always pH 6.7 and the tap-water used for the experiment was drawn from this pond.

The material employed was all three years old, with the body length varying from 20.5 to 27.5 cm. and the body weight from 150 to 285 gms. The experiment was carried on repeatedly in the course of the spring of 1924 (from March 25 to April 15). The water temperature ranged from 3°.5 to 10°.C. and the air temperature from 9°. to 20°.C.

The blood used in the experiment was diluted 3 to 101 fold. For the 3 fold dilution 1 cc. of blood was discharged into 2 cc. of saline indicator solution, and for 6 fold dilution 1 cc. of blood was added to

5 cc. of the same solution. For the 11 to 101 fold dilutions, 0.5 to 0.05 cc. of blood was added to 5 cc. of saline solution.

B. Results.

1. *pH value.* According to the results obtained from 31 experiments, the blood of carp showed a pH value of 7.61 at three fold dilution, 7.63 at 6 fold dilution, 7.60 at 11 fold dilution, 7.54 at 21 fold dilution, 7.47 at 51 fold dilution, and 7.41 at 101 fold dilution. A slight increase of alkalinity was observed when the dilution went

TABLE II.

Case No.	pH (Degree of dilution)						Temperature (C°)		pH	Fish (Dimensions)			Time (1924)		
	3 fold	6 fold	11 fold	21 fold	51 fold	101 fold	Room	Water	Water	Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	7.62	7.63	7.61	—	—	—	13.0	6.5	6.7	20.5	5.6	157	April	9	1.30 p.m.
2	7.60	7.62	7.50	—	—	—	15.0	7.7	6.7	23.5	6.5	185	,,	,,	5.30 p.m.
3	7.60	7.62	7.60	—	—	—	10.6	6.0	6.7	24.5	6.8	210	,,	,,	11.30 a.m.
4	7.54	7.60	7.57	—	—	—	10.6	6.0	6.7	23.4	6.5	185	,,	,,	Noon
5	7.70	7.74	7.74	—	—	—	10.6	6.0	6.7	26.0	6.6	230	,,	,,	12.30 p.m.
6	7.58	7.60	7.59	—	—	—	15.8	7.5	6.7	24.0	6.4	207	,,	,,	4.30 p.m.
7	7.62	7.65	7.60	—	—	—	11.8	6.8	6.7	27.5	6.7	240	,,	10	10.30 a.m.
8	7.61	7.63	7.60	—	—	—	15.0	7.2	6.7	24.0	6.5	211	,,	,,	11.30 a.m.
9	7.57	7.60	7.56	—	—	—	19.0	8.3	6.7	24.5	6.0	185	,,	,,	1.30 p.m.
10	7.62	7.64	7.63	—	—	—	20.0	9.8	6.7	20.5	5.7	150	,,	,,	4.00 p.m.
11	—	7.62	7.62	7.61	7.57	7.50	13.0	5.0	6.7	23.7	6.2	182	March	25	5.00 p.m.
12	—	7.61	7.62	7.60	7.58	7.48	13.0	4.3	6.7	25.0	6.7	225	,,	,,	5.30 p.m.
13	—	7.64	7.60	7.52	7.47	7.41	13.5	4.7	6.7	23.0	6.1	178	,,	26	11.00 a.m.
14	—	7.62	7.53	7.46	7.39	7.32	13.5	4.8	6.7	25.0	6.6	215	,,	,,	11.30 a.m.
15	—	7.62	7.56	7.55	7.47	7.31	13.5	4.8	6.7	25.0	6.5	210	,,	,,	11.50 a.m.
16	—	7.64	7.62	7.60	—	—	9.0	3.5	6.5	23.0	6.0	175	,,	27	12.30 p.m.
17	—	7.63	7.59	7.48	7.53	7.48	13.0	4.8	6.7	22.3	6.3	285	,,	31	11.00 a.m.
18	—	7.69	7.58	7.55	7.45	7.35	13.0	4.8	6.7	24.1	6.4	215	,,	,,	11.30 a.m.
19	—	7.65	7.64	7.56	7.21	7.45	13.0	4.8	6.7	22.2	6.6	190	,,	,,	11.45 a.m.
20	—	7.64	7.63	7.60	7.51	7.49	13.0	4.8	6.7	23.5	5.9	188	,,	,,	11.55 a.m.
21	—	7.65	7.63	7.60	7.60	7.50	13.2	4.8	6.7	21.0	5.8	149	,,	,,	12.10 p m.
22	—	7.57	7.52	7.51	7.49	7.47	11.2	7.9	6.7	22.0	6.1	170	April	11	11.00 a.m.
23	—	7.65	7.64	7.61	7.44	7.33	12.0	7.9	6.7	22.1	6.2	180	,,	,,	11.30 a.m.
24	—	7.35	7.35	7.28	7.25	7.20	12.0	7.9	6.7	23.1	6.1	185	,,	,,	11.40 a.m.
25	—	7.66	7.66	7.60	7.59	7.50	18.2	8.0	6.7	22.5	6.2	175	,,	,,	2.30 p.m.
26	—	7.63	7.61	7.58	7.50	—	19.5	8.0	6.7	23.8	6.3	180	,,	,,	3.00 p.m.
27	—	7.64	7.52	7.48	7.43	7.36	19.7	8.0	6.7	22.5	6.0	165	,,	,,	3.50 p.m.
28	—	7.70	7.71	7.64	7.57	7.50	11.6	8.3	6.7	22.0	5.3	145	,,	14	2.50 p.m.
29	—	7.65	7.61	7.49	7.44	7.41	12.1	8.5	6.7	20.5	6.5	175	,,	,,	3.15 p.m.
30	—	7.64	7.63	7.60	7.50	7.40	18.0	10.0	6.7	23.0	6.0	163	,,	15	5.00 p.m.
31	—	7.62	7.60	7.48	7.48	7.41	18.0	10.0	6.7	22.8	5.6	150	,,	,,	5.30 p.m.
Mean	7.61	7.63	7.60	7.54	7.47	7.41	—	—	—	23.2	6.2	189	—	—	—

The pH of carp blood in varying dilutions.

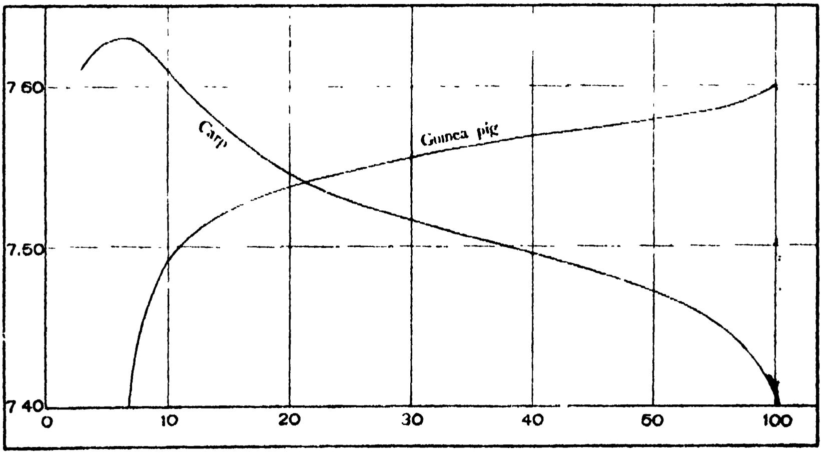
from 3 fold to 6 fold, and at 101 fold dilution the alkalinity decreased as much as 0.2 when compared with that of 3 fold dilution.

The changes of alkalinity caused by dilution are much different according as to the use of carp blood or mammalian blood. The carp blood showed a gradual decrease in its alkalinity with an increasing dilution when studied colorimetrically; the mammalian blood, on the contrary, increased its alkalinity rather rapidly with dilution, as seen in the results obtained from the studies on the blood of the guinea pig, which agree with those by CULLEN (1922), as can be recognized in the following table.

Degree of dilution	6 fold	11 fold	21 fold	51 fold	101 fold
Guinea pig	7.30 7.31	7.51 7.49	7.55 7.53	7.58 7.58	7.60 7.60
Average	7.31	7.50	7.54	7.58	7.60

The comparison of the case of the carp with that of the guinea pig shows the dilution curve seen in the annexed figure.

FIG. 1.



Ordinate, pH value.
Abscissa, degree of dilution (fold).

Six fold dilution shows the highest pH value and seems in the case of the carp blood to indicate accordingly the maximum alkalinity, so that this dilution may be taken as the optimum in this colorimetry. As a rule the higher dilution is the end reaction and becomes more indefinite, thus giving a greater value of deviation from the mean, consequently too high a dilution is not desirable.

By the 3 fold dilution the maxium alkalinity showed a pH value of 7.70, and the minimum showed 7.54, while by the dilution of 101 fold maximum alkalinity showed a pH value 7.50 and the minimum showed pH 7.20. To determine the effect of further dilution, 251 fold and 501 fold were tested, and the results were that the 251 fold dilution showed a 0.03 to 0.15 lower pH value than the 101 fold dilution, and same amount of difference was obtained from the 501 fold dilution.

Furthermore, the alkalinity of blood from the two individuals was examined at different times. As seen in the following table the results of this experiment showed some discrepancies. In one case the blood alkalinity showed a little increase while in another case it showed a distinct decrease.

Dilution / Case	pH					Date (1925)	Water-temp. (C°)	Dimensions of fishes		
	6 fold	11 fold	21 fold	51 fold	101 fold			Length (cm.)	Height (cm.)	Weight (gms.)
Case I	7.63	7.50	7.45	7.42	7.36	IV, 1, 1.00p.m.	4°.0	25.0	6.5	210
	7.64	—	7.58	7.50	7.49	IV, 11, 5.00p.m.	10°.0			
Case II	7.70	7.80	7.80	—	—	IV, 9, 4.45p.m.	7°.5	22.0	6.1	176
	7.18	7.28	7.22	—	—	IV, 11, 4.30p.m.	6°.7			

As a study of a pathological condition, I examined a fish (length 17.5 cm., height 5 cm., weight 85 gms.) about to die from Saprolegnia. The alkalinity showed a value of pH 7.80. Another fish (length 26.0 cm., height 6.4 cm., weight 220 gms.) which was weakened on account of the damaging of a gill, showed an alkalinity of pH 7.75. In a third fish (length 26.0 cm., height 6.4 cm., weight 220 gms.) which was exhausted by the loss of blood of the previous day, this value showed pH 7.50.

2. *CO_2-gas content.* To determine the CO_2-gas content and the pH value simultaneously 2 cc. of blood was collected from an individual, and one half of it was used for the estimation of the CO_2-gas, while the other half was employed for the determination of the pH value.

With ease the material could be collected from a somewhat larger fish. The fish, brought from a fish-farm, had been cultured in a small aquarium fed with tap water of pH 8.40 and temp. 5.0°C.

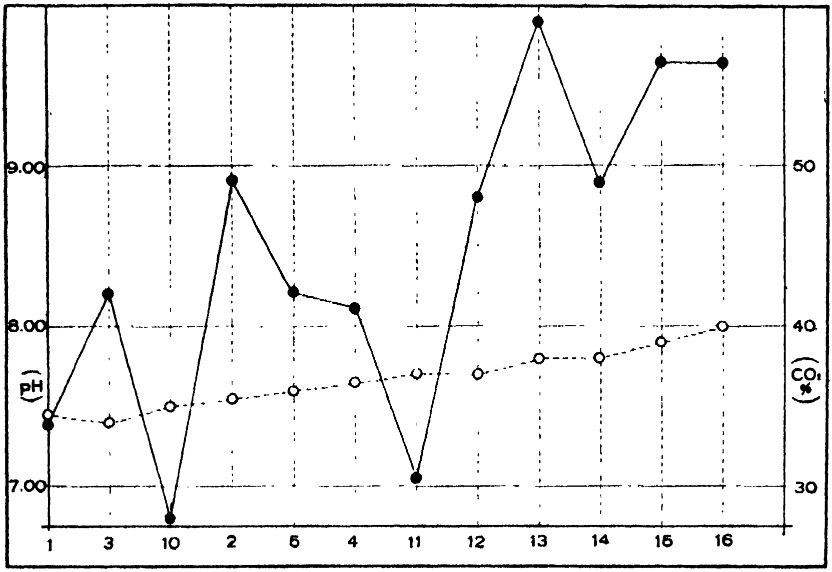
As the results of sixteen determinations, the alkalinity of the blood indicated a pH value of 7.67 in mean, showing a good coincidence with the pH value aforementioned, i. e., 7.63 at 6 fold dilution. The value of each determination varied, however, in a wider range in comparison with those of Table I. This may probably be due to the influence of the high pH value of tap water, as the pH of the blood showed the lowest value of 7.35 on an average in the experiment on the 19th, pH 7.63 on the 21th, suggesting a little increment, and finally showing the highest mean value of 7.88 on the 22th. This fact shows that the blood pH was still equilibrated with the native water on the 19th, though it acclimatized step by step to the high alkalinity of the tap water from the 21th to the 22th.

TABLE III.

Case No.	pH	CO_2-gas content vol. %	Dimensions of fishes			Time (1925)		
			Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	7.45	34.3	32.2	8.5	521	III	19	3.30 p. m.
2	7.55	49.2	31.6	8.8	537	,,	,,	4.05 p. m.
3	7.40	41.8	31.8	8.8	490	,,	,,	4.30 p. m.
4	7.65	41.4	34.5	8.6	535	,,	,,	4.55 p. m.
5	7.60	41.5	33.0	8.6	500	,,	,,	5.20 p. m.
6	—	46.4	31.7	8.7	520	,,	,,	6.00 p. m.
7	—	56.8	31.5	9.0	530	,,	,,	6.20 p. m.
8	—	48.0	31.0	8.8	537	,,	21	3.00 p. m.
9	—	39.6	33.0	8.6	500	,,	,,	3.20 p. m.
10	7.50	28.3	34.5	8.6	542	,,	,,	9.40 a. m.
11	7.70	33.0	33.5	9.4	535	,,	,,	10.40 a. m.
12	7.70	47.6	33.2	9.0	535	,,	,,	11.30 a. m.
13	7.80	58.7	32.5	8.5	430	,,	22	1.15 p. m.
14	7.80	48.8	32.0	8.8	467	,,	,,	1.50 p. m.
15	7.90	53.8	53.0	9.8	635	,,	,,	2.30 p. m.
16	8.00	58.6	31.5	9.5	535	,,	,,	3.20 p. m.
Mean	7.67	45.2	—	—	—	—	—	—

As can be seen in the above table, the CO_2-gas content of the blood of the carp fluctuated in a range of 28.3 vol. % to 58.7 vol. % with the mean value of 45.2 vol. %. According to this value the CO_2-gas content of the blood is in this case about 90% of the human venous blood and almost equal to that of the arterial blood. Seeing the relation between the pH and the CO_2-gas content, we are struck by the parallel increase and decrease of both the values. When we arrange each determination according to the rising value of pH, the increase of the CO_2-gas content can easily be seen (Fig. 2). It is thus obvious that both the pH and the CO_2-gas content showed a little deviation from the mean value. This is because the fish was about to become acclimatized to the high alkalinity of tap water.

FIG. 2.



Relation between the pH and the CO_2-content.
Broken curve — pH
Continued curve — CO_2%
No. on left side — pH
No. on right side — % of CO_2
No. on abscissa — No. of case

EXPERIMENT III. Rainbow trout (*Salmo iridius*).

A. Case history.

The rainbow trout, *Salmo iridius*, is a fresh-water Salmonid fish which is cultured in the pond. The present investigation was undertaken at the hatchery of Aisaka, Aomori prefecture, where the fish is reared and cultured for economic purposes. The fish employed were all 4 to 5 years old and measured 36.4 to 49.9 cm. in length, 8.1 to 10.6 cm. in height, and 555 to 1600 gms. in weight. 3 cc. of blood was drawn from one fish, 2 cc. of which was used for the determination of the hydrogen ion concentration, and the rest for the estimation of the CO$_2$-gas content. The blood was not oxalated in all the cases, as there is a tendency to cause haemolysis by the addition of potassium oxalate.

In as much as the fish are very gentle, the bleeding could easily be carried out by a heart puncture, some one else holding the abdomen towards the operator. The fish never died after the operation if it was replaced in the pond at once.

B. Results.

1. *Hydrogen Ion Concentration.* As far as concern the present 11 determinations, the blood of the rainbow trout showed a pH value of 7.28 at 6 and 21 fold dilution, 7.29 at 11 fold dilution, and 7.27 at 51 and 101 fold dilution. As a marked distinction from the case of the carp, the blood of the rainbow trout showed but only a slight change according to dilution (See Fig. 3). Although it shows a slight increase at the 11 fold dilution and a slight decrease at the 51 to 101

TABLE IV.

Case No.	pH Degree of dilution					CO$_2$ content vol. %	Dimensions of fishes			Time (1925)		
	6 fold	11 fold	21 fold	51 fold	101 fold		Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	7.35	7.30	7.30	7.30	7.30	19.7	36.4	8.2	555	Apr.	28th	11.30 a. m.
2	7.43	7.43	7.43	7.43	7.43	19.5	46.0	8.8	704	,,	,,	12.30 p. m.
3	7.35	7.40	7.43	7.42	7.38	22.9	49.6	10.6	1600	,,	,,	1.00 p. m.
4	7.30	7.29	7.29	7.29	7.29	24.8	45.5	10.0	1110	,,	,,	2.00 p. m.
5	7.35	7.32	7.30	7.30	7.30	31.1	49.9	10.0	1074	,,	,,	3.00 p. m.
6	7.35	7.33	7.31	7.30	7.30	27.3	44.5	9.3	852	,,	,,	3.25 p. m.
7	7.35	7.38	7.34	7.30	7.32	26.4	37.8	8.2	555	,,	,,	3.40 p. m.
8	7.15	7.20	7.18	7.18	7.20	27.3	48.4	10.0	1185	,,	,,	4.25 p. m.
9	7.15	7.17	7.17	7.15	7.15	26.1	36.4	8.2	555	,,	,,	4.45 p. m.
10	7.25	7.27	7.26	7.25	7.25	21.5	38.1	8.1	667	,,	,,	5.10 p. m.
11	7.05	7.05	7.05	7.05	7.05	23.6	39.4	8.7	667	,,	,,	5.30 p. m.
Mean	7.28	7.29	7.28	7.27	7.27	24.6	—	—	—	—	—	—

The pH and the CO$_2$-gas content of the Blood of Rainbow trout.

fold dilutions, it resembles *Lateolabrax*, *Pagrosomus*, and *Pseudomonacanthus*, as will be stated later, because it shows no marked change by the dilution.

2. *CO_2-gas content.* The CO_2-gas content ranged between 19.5 vol. % and 31.1 vol. % indicating a mean value of 24.6 vol. %, which corresponds to about 54.0% of that of the carp and about 49.0% of that of the human venous blood. There seems, in this case, to be no definite relation between the pH and the CO_2-gas content as seen from the fact that the CO_2-gas content changes considerably even for the same pH value; for instance, the CO_2-gas content was 19.7 vol. % in the case of No. 1, whereas it showed 31.1 and 27.3 vol. % in the case of No. 5 and No. 6 for the same pH value of 7.35. A low pH value for a high CO_2-gas content and a high value for a low CO_2-gas content may be attributed to the tension of the free carbon dioxide of the blood. When blood has a high tension of free carbon dioxide, the pH may be low in spite of its high bicarbonate content, and when the carbon dioxide tension is held low, the pH may be high even though the bicarbonate content is relatively low. COLLIP (1920) gives a similar statement regarding the relation of the pH value and the bicarbonate content of fish blood and of sea water.

EXPERIMENT IV. Ugui (*Leuciscus hakuensis*).

A. Case history.

Ugui is one of the most familiar fishes which is caught in the vicinity of our station, mainly in the spring. It lives in fresh water as well as in sea water and ascends the rivers from April to July to spawn. The fish employed in the present experiment were collected by means of a trap net set in the small river at a place 100 meters up from the estuary. Prior to the experiment, however, they were kept in a sea water aquarium about three hours. The fish were all three years old and measured 32.0 to 35.0 cm. in length, 6.5 to 8.0 cm. in height, and 438 to 506 gms. in weight. Three or four cc. of blood was bled from one fish, of which 2 cc. was employed for the determination of the hydrogen ion concentration, and the rest for the estimation of the CO_2-gas content and capacity. Bleeding was done as soon as the fish had been beaten to death.

B. Results.

1. *Hydrogen ion concentration.* The hydrogen ion concentration of the blood of this fish indicates just a value medium to that of *Cyprinus* and *Salmo,* showing a pH value of 7.45 in 6 fold dilution, 7.44 in 11 fold dilution, 7.42 in 21 fold dilution, 7.39 in 51 fold dilution, and 7.35 in 101 fold dilution. A decrease of alkalinity by increasing the dilution is not so distinct as in *Cyprinus ;* on the other hand it is much more obvious than in *Salmo.*

2. *CO_2-gas content and capacity.* According to the present determinations, the CO_2-gas content of the blood of this fish ranges between 16.4 to 20.2 vol. % showing an average value of 18.3 vol. %, as can be seen in the next table. The CO_2-gas capacity amounted to 25.3 to 27.8 vol. % with an average value of 26.6 vol. %, showing a difference of 8.3 vol. % as compared with the CO_2-gas content. The saturated portion, i. e., the difference between the capacity and the content, was 7.9 vol. % in the minimum, 11.4 vol. % in the maximum, and 8.3 vol. % on an average. The unsaturated percentage was 31.2% in the minimum, 41.0% in the maximum, and 31.2% on the average.

TABLE V.

Case No.	pH					CO_2		Dimensions of fishes			Time (1925)		
	Degree of dilution												
	6 fold	11 fold	21 fold	51 fold	101 fold	Content vol. %	Capacity vol. %	Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	7.22	7.20	7.18	7.15	7.12	19.3	—	35.0	7.0	383	Apr.	28th	2.30 p.m.
2	7.70	7.70	7.70	7.70	7.60	17.4	25.3	33.0	8.0	506	,,	,,	3.00 p.m.
3	7.56	7.56	7.55	7.47	7.43	18.1	—	33.0	7.5	395	,,	29th	9.30 a.m.
4	7.30	7.30	7.27	7.20	7.20	16.4	27.8	32.0	7.0	348	,,	29th	10.00 a.m.
5	7.47	7.46	7.42	7.41	7.41	20.2	—	34.0	6.5	399	,,	,,	10.30 a.m.
Mean	7.45	7.44	7.42	7.39	7.35	18.3	26.6	—	—	—	—	—	—

The pH and the CO_2-gas content of the blood of Ugui.

EXPERIMENT V. Buri (*Seriola aureovittata*).

A. Case history.

The fish employed for this experiment were caught in the vicinity of our station in the beginning of August, and since then they were cultured in the aquarium of the station until used. The nature of this fish is migratory and it is pelagic in habit and enters Aomori Bay usually about the middle of June. It constitutes an important part of the summer fishing.

As this fish flounders violently when taken from the water, the blood can not be taken directly from the living creature. It was killed beforehand, and the puncture was immediately undertaken. The blood taking must be finished within 30 seconds and the dilution also must be finished within 1 minute, to prevent the coagulation of the blood. The pipette used for the blood taking was previously filled with paraffin oil.

B. Results.

1. *Hydrogen ion concentration.* The hydrogen ion concentration of the blood of the Buri showed pH 7.40 in 6 fold dilution, pH 7.35 in 11 fold dilution, pH 7.32 in 21 fold, and pH 7.25 in 101 fold dilution. We see in the following table that the alkalinity decreases distinctly in the dilutions of 6 to 21 fold, while it remains constant in the dilutions of 51 to 101 fold.

TABLE VI.

Case No.	pH					Room temp.	Dimensions of fishes			Time (1925)		
	Degree of dilution											
	6 fold	11 fold	21 fold	51 fold	101 fold		Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	7.50	7.40	7.40	7.28	7.28	19°.7	36.0	7.5	485	Oct.	16	9.00 a. m.
2	7.35	7.30	7.27	7.24	7.24	20°.0	36.5	8.0	525	,,	16	9.20 a. m.
3	7.30	7.30	7.28	7.20	7.20	20°.0	36.5	7.8	505	,,	16	9.40 a. m.
4	7.43	7.40	7.30	7.28	7.28	20°.0	37.2	8.2	610	,,	16	10.00 a. m.
5	7.43	7.35	7.33	7.25	7.25	20°.0	36.3	7.7	500	,,	16	10.00 a. m.
Mean	7.40	7.35	7.32	7.25	7.25	20°.0	36.5	7.8	525	—	—	—

The pH of the blood of Buri in varying dilutions.

EXPERIMENT VI. Umadurahagi (*Pseudomonacanthus modestus*).

A. Case history.

The present species is one of the commonest fish which is caught in our vicinity during June and July. The fish used in this experiment were caught in July and cultured in our aquarium until September.

Since this fish is very gentle and does not flounder when taken out of water, the puncture can easily be finished. The fish, however, is not large enough to get over 1 cc. of blood from one individual, so it is necessary to employ lower dilutions of 11 to 101 fold instead of 6 to 101 fold used in the foregoing cases.

The size of the fish employed for the experiment was 24.2 to 28.5 cm. in length, 7.5 to 9.0 cm. in breadth, and 140 to 260 gms. in weight. The blood not only is lighter red in colour, but is less viscous than that of the Carp and the Buri.

B. Results.

1. *Hydrogen ion concentration.* The hydrogen ion concentration of the blood was scarcely influenced by dilution. By the dilutions of 11 to 21 fold it showed a definite value of pH 7.49, which decreased only a little by further dilution, for instance, 7.48 at 51 fold dilution and pH 7.47 at 101 fold dilution.

TABLE VII.

Case No.	pH				Room temp.	Dimensions of fishes			Time (1925)		
	Fold of dilution										
	11 fold	21 fold	51 fold	101 fold		Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	7.50	7.50	7.50	7.47	20°.1	28.0	9.0	260	Oct.	16	12.20 p.m.
2	7.50	7.48	7.47	7.47	20°.0	24.2	7.5	167	,,	16	12.45 p.m.
3	7.42	7.42	7.42	7.42	20°.0	28.5	8.5	247	,,	16	2.10 p.m.
4	7.54	7.55	7.53	7.53	19°.0	25.3	7.5	140	,,	16	4.30 p.m.
5	7.49	7.50	7.49	7.48	18°.9	25.6	7.6	140	,,	16	4.50 p.m.
Mean	7.49	7.49	7.48	7.47	19°.6	26.8	8.0	191	—	—	—

The pH of the blood of Umadurabagi in varying dilutions.

2. *CO_2-gas content and capacity.* Together with the estimation of the CO_2 the pH value was tested also. For this purpose 1.5 cc. of blood was collected from a fish, of which 1 cc. was used for the CO_2 determination and the remaining 0.5 cc. for the estimation of the pH value.

As seen in the Table VIII, the blood of this fish contains 8.1 to 11.9 vol. % of CO_2-gas, showing a mean value of 9.7 vol. %. The pH value corresponding to these gas contents was 7.47 to 7.52 and 7.48 on an average. The blood of high pH value does not always

TABLE VIII.

Case No.	pH (11 fold)	CO_2-content vol. %	Dimensions of fishes			Time (1925)		
			Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	7.50	9.9	28.0	8.8	247	July	30	7.30 a. m.
2	7.49	8.1	30.0	9.1	293	,,	30	7.50 a. m.
3	7.51	11.9	24.6	7.8	147	,,	30	8.25 a. m.
4	7.47	8.8	25.0	7.7	173	,,	30	7.00 p. m.
5	7.52	8.7	26.0	7.9	188	,,	30	7.20 p. m.
6	7.47	8.7	26.2	7.9	198	,,	30	7.40 p. m.
7	7.52	8.8	25.0	7.6	169	,,	30	8.00 p. m.
8	7.48	8.7	29.0	9.0	215	,,	30	8.20 p. m.
9	7.51	9.9	30.0	9.5	295	,,	30	8.40 p. m.
10	7.47	9.9	28.5	9.0	230	,,	30	9.00 p. m.
11	7.32	11.4	29.0	8.8	263	,,	30	9.20 p. m.
12	7.50	9.9	29.5	9.1	240	,,	31	7.00 p. m.
13	7.52	9.4	24.5	7.1	158	,,	31	7.30 p. m.
14	7.49	8.8	25.9	7.8	191	,,	31	7.50 p. m.
15	7.51	10.9	24.4	7.0	165	,,	31	8.15 p. m.
16	7.50	9.3	28.8	8.8	200	,,	31	8.35 p. m.
Average	7.48	9.7	27.1	8.3	211	—	—	—

The pH and the CO_2-gas content of the blood of Umadurahagi.

contain much CO_2-gas (see, for instance, case No. 16) and such a relation between a high pH value and a high CO_2-gas content may be regarded as the tension of the free carbon dioxide.

EXPERIMENT VII. Suzuki (*Lateolabrax japonicus*).

A. Case history.

The present species is caught from June to October all along the coast of Aomori Bay. The individuals used for this experiment were kept about a month in our aquarium and were from 32.0 to 68.5 cm. in length. The blood collection went on quickly, beginning within 20 seconds after the fish was hit on its head with a club, and was finished within 1 minute, as this fish is very floundering. The size of the fish used was 32.0 to 68.5 cm. in length, and the individuals over 50.0 cm. gave so much blood that 2 cc. could be collected twice or thrice in succession. In the present case, the pH value was estimated twice or thrice on one and the same individual at an interval of 30 minutes; the variation of the pH after the death of the animal was thus made out.

B. Results.

1. *Hydrogen ion concentration.* From an inspection of the following table it is obvious that the blood does not alter, in this case, its hydrogen ion concentration by promoting the dilution from 6 to 51 fold, keeping the pH value of 7.35. By the dilution of 101, however, its hydrogen ion concentration decidedly decreases to a pH value of 7.38. After the death of the animal the hydrogen ion decreases; for instance, the pH value indicates 7.21 in the case of 6 to 51 fold dilutions and 7.24 in the dilution of 101 fold (Table IX). The increase of the alkalinity following the dilution of 101 fold, though faint, is distinctly recognizable, even 30 minutes after death.

TABLE IX.

Case No.	pH					pH after 30 minutes					Room temp.	Dimensions of fishes			Time (1925)		
	Degree of dilution					Degree of dilution											
	6 fold	11 fold	21 fold	51 fold	101 fold	6 fold	11 fold	21 fold	51 fold	101 fold		Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	7.25	7.25	7.25	7.25	7.27	6.80	6.80	6.80	6.80	6.80	15°.3	68.5	14.3	2900	Oct.	22	11.00 a. m.
2	7.36	7.36	7.36	7.36	7.38	7.05	7.05	7.05	7.05	7.07	15°.4	34.0	6.9	305	,,	22	12.30 p. m.
3	7.40	7.40	7.40	7.40	7.45	7.10	7.10	7.10	7.10	7.15	14°.6	53.5	9.5	1060	,,	23	10.30 p. m.
4	7.40	7.39	7.38	7.37	7.38	7.00	7.00	7.00	7.00	7.05	15°.3	32.0	6.5	275	,,	23	2.30 p. m.
5	7.35	7.35	7.35	7.35	7.40	7.10	7.10	7.10	7.10	7.13	15°.0	52.0	9.0	925	,,	23	3.45 p. m.
Mean	7.35	7.35	7.35	7.35	7.38	7.21	7.21	7.21	7.21	7.24	15°.1	—	—	—	—	—	—

The pH of the blood of Suzuki in varying dilutions.

Since 101 fold dilution increased the alkalinity, 500 fold dilution was also tested to examine whether or not a further dilution increases the alkalinity. The result was that this dilution showed a little lower alkalinity as compared with the result obtained in the case of the 6 to 51 fold dilutions, indicating a pH value of 7.38.

EXPERIMENT VIII. Tai (*Pagrosomus major*).

A. Case history.

This fish was kept living about two months in the aquarium after its captivity in July in the vicinity of our laboratory and was 23.3 to 44.5 cm. in length. The blood colour was dark, like that of the Carp and the Suzuki.

B. Results.

1. *Hydrogen ion concentration.* The hydrogen ion concentration of the blood of Tai showed an almost constant value of pH 7.35 when it was diluted 6 to 51 fold. In this respect this fish resembles the Suzuki already mentioned. The difference from that of Suzuki, however, consists in the pH value of blood which shows a slight decrease of alkalinity when diluted to 101 fold.

In the case of 500 fold dilution the alkalinity was pH 7.29 in the individual No. 3 of the following table.

TABLE X.

Case No.	pH Degree of dilution 6 fold	11 fold	21 fold	51 fold	101 fold	Room temp. (C°)	Water temp (C°)	pH of Water	Atmos. press.	Dimensions of fishes Length (cm.)	Height (cm.)	Weight (gms.)
1	7.38	7.39	7.38	7.38	7.36	13°.5	17.5	8.10	761	25.3	8.5	250
2	7.40	7.40	7.40	7.39	7.34	14°.8	17.5	8.10	761	37.0	12.2	845
3	7.30	7.32	7.33	7.33	7.30	11°.5	17.5	8.10	766	26.2	8.8	250
4	7.26	7.26	7.26	7.26	7.24	11°.8	17.5	8.10	766	23.3	7.5	185
5	7.40	7.40	7.40	7.40	7.40	12°.0	17.5	8.10	766	44.5	14.2	1335
Mean	7.35	7.35	7.35	7.35	7.33	12°.7	17.5	8.10	764	—	—	—

The pH of the blood of Tai in varying dilutions.

EXPERIMENT IX. Sironamako (*Caudina chilensis*).

A. Case history.

Caudina is a kind of Holothuria found all the year around in the shore of Aomori Bay about 20 cm. deep in the sand. The body is spindle shaped, having a long tail like appendage on one end, which enables the animal to respire. The animal gave 5 to 15 cc. of dark red blood which was not so viscous as that of higher animals, showing a density of 1.0275 and containing numerous red fusiform corpuscles provided with two spines on both terminals. When the fresh blood was left standing 2 or 3 hours in a vessel, the corpuscles deposited on the bottom, being separated from the plasma weich did not clot, apparently containing no fibrin at all. The plasma varies more or less in colour according to individuals; it was however, light yellow in most cases, while in some cases it was almost colourless and in another case deep yellow. The specimen I employed were 11.5 to 19.5 cm. in length, 2.7 to 3.5 cm. in diameter, and 40 to 90 gms. in weight.

B. Results.

1. *Hydrogen ion concentration.* The plasma of *Caudina* showed a pH value of 7.79 in the undiluted state. It was, however, of pH 7.63

TABLE XI.

Case No.	pH Degree of dilution						Room temp. (C°)	Water temp. (C°)	Atmos. press. (mm.)	pH of Water	Dimensions of animals			Time (1925)		
	0 fold	6 fold	11 fold	21 fold	51 fold	101 fold					Length (cm.)	Diameter (cm.)	Weight (gms.)	Month	Date	Hour
1	—	7.30	7.25	7.20	7.15	7.10	20.7	20.0	763	8.10	13.0	3.1	40	X	7	Noon.
2	—	7.70	7.68	7.50	7.45	7.40	21.5	20.5	760	8.10	14.0	3.0	58	X	7	4.20 p.m.
3	—	7.65	7.65	7.60	7.50	7.40	21.3	20.7	763	8.10	17.0	3.0	70	X	7	5.50 p.m.
4	—	7.70	7.70	7.64	7.55	7.55	21.0	19.5	760	8.12	14.9	2.9	60	X	11	3.30 p.m.
5	—	7.73	7.74	7.74	7.70	7.70	21.3	19.5	760	8.12	16.8	3.2	75	X	11	3.45 p.m.
6	—	7.72	7.72	7.67	7.64	7.50	21.3	19.5	760	8.12	15.0	3.5	65	X	11	4.00 p.m.
7	—	7.61	7.61	7.61	7.55	7.55	21.3	19.5	760	8.12	15.2	3.0	59	X	11	4.15 p.m.
8	—	7.62	7.62	7.62	7.54	7.54	21.3	19.5	760	8.12	15.5	2.9	50	X	11	4.30 p.m.
9	—	7.70	7.70	7.68	7.64	7.60	13.5	16.8	766	8.14	16.0	2.8	68	X	26	8.00 p.m.
10	—	7.46	7.45	7.45	7.40	7.39	13.5	16.8	766	8.14	16.0	3.0	90	X	26	8.15 p.m.
11	—	7.55	7.52	7.53	7.53	7.49	13.5	16.8	766	8.14	15.2	3.3	88	X	26	8.30 p.m.
12	—	7.31	7.32	7.35	7.40	7.41	13.6	16.8	766	8.14	15.0	3.2	73	X	26	8.45 p.m.
13	—	7.74	7.74	7.72	7.70	7.68	13.6	16.8	766	8.14	16.2	3.3	83	X	26	9.00 p.m.
14	—	7.70	7.68	7.66	7.64	7.65	15.0	16.6	764	8.12	16.1	2.9	80	X	27	10.30 p.m.
15	—	7.69	7.69	7.63	7.50	7.45	14.7	16.6	764	8.12	15.0	3.1	75	X	27	11.00 p.m.
16	—	7.70	7.70	7.68	7.61	7.60	14.8	16.6	764	8.12	14.8	3.0	70	X	27	11.20 p.m.
17	—	7.65	7.65	7.62	7.50	7.50	14.8	16.6	764	8.12	14.8	2.7	61	X	27	11.40 p.m.
18	—	7.80	7.79	7.72	7.55	7.55	15.2	16.6	763	8.12	15.9	3.1	68	X	27	1.12 p.m.
19	7.85	—	—	—	—	—	19.0	5.5	758	8.17	14.0	3.0	59	III	12	3.30 p.m.
20	8.00	—	—	—	—	—	19.0	5.5	753	8.17	14.9	2.9	63	III	12	3.50 p.m.
21	7.85	—	—	—	—	—	19.0	5.5	753	8.17	14.8	3.0	65	III	12	4.10 p.m.
22	7.90	—	—	—	—	—	19.0	5.5	753	8.17	15.0	3.0	71	III	12	4.30 p.m.
23	8.00	—	—	—	—	—	15.0	6.9	759	8.17	19.5	3.5	83	III	15	3.00 p.m.
24	7.85	—	—	—	—	—	15.0	6.9	759	8.17	16.8	3.3	95	III	15	3.20 p.m.
25	8.00	—	—	—	—	—	15.0	7.0	759	8.17	13.0	3.2	88	III	15	3.40 p.m.
26	7.85	—	—	—	—	—	15.0	7.0	759	8.17	15.5	3.3	55	III	15	3.55 p.m.
27	7.85	—	—	—	—	—	15.0	7.2	759	8.17	12.5	3.4	63	III	15	4.10 p.m.
28	8.00	—	—	—	—	—	15.0	7.2	759	8.17	14.0	2.8	55	III	15	4.25 p.m.
29	7.90	—	—	—	—	—	15.0	7.3	759	8.17	11.5	3.1	45	III	15	4.45 p.m.
30	7.80	—	—	—	—	—	15.0	7.4	759	8.17	12.0	3.2	50	III	15	5.00 p.m.
31	7.80	—	—	—	—	—	21.1	20.3	763	8.07	15.8	3.5	70	VII	28	1.00 p.m.
32	7.70	—	—	—	—	—	21.7	20.3	763	8.07	12.1	3.2	54	VII	28	3.00 p.m.
33	7.80	—	—	—	—	—	21.7	20.5	763	8.07	16.8	3.5	89	VII	28	3.45 p.m.
34	7.90	—	—	—	—	—	21.8	20.5	763	8.07	13.0	3.2	63	VII	28	4.00 p.m.
35	7.80	—	—	—	—	—	21.8	20.5	763	8.07	16.0	3.4	90	VII	28	4.30 p.m.
36	7.70	—	—	—	—	—	21.8	20.5	763	8.07	17.0	3.7	81	VII	28	8.00 p.m.
37	7.75	—	—	—	—	—	21.0	19.7	763	8.07	13.8	3.2	60	VII	28	
Mean	7.79	7.68	7.62	7.59	7.53	7.50	—	—	—	—	—	—	—	—	—	—

The pH of the blood of Sironamako in varying dilutions.

in 6 fold dilution, of 7.62 in 11 fold dilution, of 7.59 in 21 fold dilution, of 7.53 in 51 fold dilution, and of 7.50 in 101 fold dilution. Thus its alkalinity decreases with increasing dilution. The most sudden decrease occurs, however, within the 5 fold dilution, while further dilutions decrease the pH value very slowly.

Not only this but also the alkalinity of the plasma decreases in the course of the time left standing. The pH value 20 hours after collection was obviously decreased, as compared with that after 3 hours, as seen in the cases from No. 1 to No. 5 of the Table XII. Especially the 6 fold dilution in No. 1 showed a pH 7.70 at the third

TABLE XII.

Case No.	Time	pH Degree of dilution 6 fold	 11 fold	 21 fold	 51 fold	 101 fold
1	after 3 hours	7.70	7.70	7.64	7.55	7.55
	„ 20 „	7.10	7.15	7.22	7.22	7.38
2	after 3 hours	7.73	7.74	7.74	7.70	7.70
	„ 20 „	7.35	7.40	7.38	7.38	7.37
3	after 3 hours	7.72	7.72	7.67	7.64	7.50
	„ 20 „	7.20	7.22	7.23	7.38	7.38
4	after 3 hours	7.61	7.61	7.61	7.55	7.55
	„ 20 „	7.26	7.27	7.30	7.30	7.30
5	after 3 hours	7.62	7.62	7.62	7.54	7.54
	„ 20 „	7.10	7.18	7.22	7.22	7.24
Mean	after 3 hours	7.67	7.68	7.66	7.60	7.57
	„ 20 „	7.20	7.24	7.27	7.30	7.33
6	after 3 hours	7.70	7.70	7.68	7.64	7.60
	„ 17 „	7.64	7.65	7.63	7.63	7.62
	„ 38 „	7.30	7.40	7.38	7.50	7.53
7	after 3 hours	7.46	7.45	7.45	7.40	7.39
	„ 17 „	7.41	7.41	7.40	7.40	7.40
	„ 38 „	7.40	7.40	7.40	7.40	7.40
8	after 3 hours	7.55	7.52	7.53	7.53	7.49
	„ 17 „	7.46	7.45	7.45	7.45	7.44
	„ 38 „	7.32	7.40	7.41	7.41	7.50

Case No.	Time	pH Degree of dilution 6 fold	 11 fold	 21 fold	 51 fold	 101 fold
9	after 3 hours	7.31	7.32	7.35	7.40	7.41
	„ 17 „	7.20	7.21	7.24	7.25	7.24
	„ 38 „	7.19	7.20	7.26	7.30	7.40
10	after 3 hours	7.74	7.74	7.72	7.70	7.68
	„ 17 „	7.70	7.70	7.69	7.65	7.60
	„ 38 „	7.60	7.60	7.60	7.60	7.60
Mean	after 3 hours	7.55	7.55	7.55	7.53	7.51
	„ 17 „	7.48	7.48	7.48	7.48	7.46
	„ 38 „	7.36	7.40	7.41	7.44	7.49
11	after 3 hours	7.70	7.68	7.66	7.64	7.65
	„ 24 „	7.37	7.39	7.40	7.40	7.42
12	after 3 hours	7.69	7.69	7.63	7.50	7.45
	„ 24 „	7.50	7.52	7.53	7.53	7.52
13	after 3 hours	7.70	7.70	7.68	7.61	7.60
	„ 24 „	7.58	7.59	7.59	7.58	7.59
14	after 3 hours	7.65	7.65	7.62	7.50	7.50
	„ 24 „	7.50	7.54	7.56	7.56	7.56
15	after 3 hours	7.80	7.79	7.72	7.55	7.55
	„ 24 „	7.62	7.65	7.65	7.64	7.63
Mean	after 3 hours	7.71	7.72	7.66	7.56	7.55
	„ 24 „	7.51	7.53	7.55	7.54	7.54

Table showing the variation of pH due to the lapse of time.

hour, while it showed 7.10 at the twentieth hour. The same relation reappeared in the cases from No. 6 to No. 10 and the cases from No. 11 to No. 15. It is, therefore, evident that at the third hour the more the dilution advances, the lesser the alkalinity, while at the twentieth hour the alkalinity rises with increasing dilution.

1. *CO_2-gas content and capacity.* The blood can easily be taken from this animal by inserting the syringe needle into the body cavity. When the skin of the animal is cut open under paraffin oil in a vessel, a quantity of blood was easy to collect in the bottom of the vessel without touching the air. An individual usually gave from 5 to 15 cc. of blood, but from a large individual whose body weighs about 50 gms., 20 cc. of blood could be collected. It is, therefore, not difficult to estimate the pH value, the CO_2-gas content and the capacity with the

TABLE XIII.

Case No.	Undiluted plasma pH	CO_2-gas		Unsaturated portion	Unsaturated percentage	Dimensions of animals			Time (1925)		
		Content vol. %	Capacity vol. %			Length (cm.)	Diameter (cm.)	Weight (gms.)	Month	Date	Hour
1	8.00	10.2	11.0	0.8	7.3	19.5	3.5	83	III	15	3.00 p. m.
2	7.85	10.7	11.2	0.5	4.5	16.8	3.3	95	III	15	3.20 p. m.
3	8.00	7.4	11.3	3.9	34.5	18.0	3.2	88	III	15	3.40 p. m
4	7.85	9.9	12.0	2.1	17.5	15.5	3.3	55	III	15	3.55 p. m.
5	7.85	10.1	10.2	0.1	1.0	12.5	3.4	63	III	15	4.10 p. m.
6	8.00	8.0	10.3	2.3	22.3	14.0	2.8	55	III	15	4.25 p m.
7	7.90	7.4	10.3	2.9	28.2	15.5	3.1	45	III	15	4.45 p. m.
8	7.80	7.4	10.2	2.8	27.5	12.0	3.2	50	III	15	5.00 p. m.
9	7.80	9.1	11.7	2.6	22.2	14.7	3.0	68	III	29	2.00 p. m.
10	7.75	8.5	12.4	3.9	31.4	12.5	3.1	58	III	29	3.00 p. m.
11	7.78	8.1	12.1	4.0	33.1	19.3	3.5	80	III	29	4.00 p m.
12	7.85	8.0	12.0	4.0	33.3	13.5	3.2	45	III	29	5.00 p. m.
13	7.90	8.0	12.4	4.4	35.5	18.1	3.1	75	III	29	6.00 p. m.
14	7.70	7.5	11.4	3.9	34.2	19.3	3.6	90	IV	16	3.00 p. m.
15	7.70	6.6	9.4	2.8	30.0	19.3	3.5	98	IV	16	3.40 p. m.
16	7.83	7.4	11.9	4.5	37.7	18.9	3.4	91	IV	16	4.15 p. m.
17	7.80	7.5	11.0	3.5	31.8	16.9	3.2	90	IV	16	5.00 p. m.
18	7.80	6.7	11.0	4.3	39.1	17.8	3.3	89	IV	16	5.40 p. m.
19	7.80	10.8	10.9	0.1	0.9	13.5	3.5	70	VII	28	1.00 p. m.
20	7.70	8.5	10.5	2.0	19.0	12.1	3.2	54	VII	28	2.00 p. m.
21	7.80	8.5	10.9	2.4	18.3	16.8	3.5	89	VII	28	3.00 p. m.
22	7.90	9.8	11.6	1.8	15.5	13.0	3.2	63	VII	28	3.45 p. m.
23	7.80	8.6	11.3	2.7	23.9	16.0	3.4	90	VII	28	4.00 p. m.
24	7.70	9.3	11.3	2.0	17.6	17.0	7.8	81	VII	28	4.30 p. m.
25	7.75	8.9	11.0	2.1	19.1	13.8	3.2	60	VII	28	8.00 p. m.
Mean	7.82	8.5	11.2	2.7	23.4	15.8	3.4	73	—	—	—

The pH CO_2-content and capacity of the blood of Sironamako.

blood from one and the same individual. The experiment was carried out with the whole blood, as well as with the plasma alone, and the results from both the cases were compared.

The CO_2-gas content of the blood of *Caudina* ranged from 6.6 to 10.8 vol. % with an average value of 8.5 vol. %. The CO_2-gas capacity attained 9.4 to 12.4 vol. % with an average value of 11.2%. The difference of the mean value of the CO_2-gas content and the capacity showed 2.7%. The unsaturated portion, i. e., the difference of the capacity and the content in each determination, showed a minimum of 0.1 cc. and a maximum of 4.3 cc. The proportion of the unsaturated portion to the CO_2-gas capacity, i.e., unsaturated percentage, indicates 0.9 to 39.1% with an average of 23.4%.

As can be seen in the following table, the CO_2-gas content of whole blood showed 8.34 vol. %, while the plasma was 8.55 vol. % as a mean of 10 determinations, surpassing the former as much as 0.21 vol. %. The result of the analysis showed that the whole blood is less variable than the plasma, the probable error of the latter (0.43) being much larger than that of the former.

TABLE XIV.

Case No.	CO_2-gas content		Dimensions of animals			Time (1925)		
	Whole blood	Plasma	Length (cm.)	Diameter (cm.)	Weight (gms.)	Month	Date	Hour
1	8.1	7.8	15.1	3.6	61	VIII	21	3.45 p. m.
2	8.2	8.3	15.0	3.4	60	VIII	21	4.10 p. m.
3	8.1	8.1	15.0	3.5	57	VIII	21	4.30 p. m.
4	8.2	8.5	17.3	3.6	79	VIII	21	4.50 p. m.
5	8.2	9.0	14.0	2.9	43	VIII	22	5.20 p. m.
6	8.2	9.8	16.5	3.0	47	VIII	22	2.05 p. m.
7	8.9	8.9	16.0	3.0	47	VIII	22	2.35 p. m.
8	8.4	7.6	17.0	2.2	44	VIII	22	3.00 p. m.
9	8.3	8.7	13.2	2.5	32	VIII	22	3.20 p. m.
10	8.8	8.8	17.0	3.0	60	VIII	22	3.45 p. m.
Mean	8.34	8.55	—	—	—	—	—	—

A Comparison of the CO_2-content of the whole blood and that of the plasma.

According to the present determination the blood of *Caudina* showed pH 7.82, CO_2-gas content 8.50 vol. %, and CO_2-gas capacity 11.20 vol. % as the normal state. As to the relation between the pH value and the CO_2-gas content it is different from that in the higher animals in which the pH increases or decreases regulary with the relative amount of free carbon dioxide and bicarbonate in the blood, while in *Caudina* the CO_2-gas content varies in a narrow range above or under the average value 8.5 vol. %. The pH varies in a wide range of pH 7.70–8.50. This may probably be due to the varying tension of the free carbon dioxide in the blood.

As the variation of the pH signifies the result of uncompensated acidosis or alkalosis in higher animals, the CO_2-gas content must vary according to the variation of the pH value, provided the tension of the free carbon dioxide remains constant. In *Caudina* the CO_2-gas content shows no regular variation which corresponds to the change of the pH value, certifying the fact that there are such varying quantities of free CO_2 as to often disturb the acid base equilibrium of the blood, thus changing the pH value.

EXPERIMENT X. Akagai (*Arca inflata*).

A. Case history.

Arca is a large edible bivalve found in abundance in Aomori Bay throughout the year. The red coloured blood of this mollusca affords nice material for the student of haemology, especially in our station on account of the ease of its collection. The individuals I employed were collected during August and were 12.3 to 14.7 cm. in length and 36.7 to 68.5 gms. in weight. In those over 300 gms. it was by no means difficult to collect as much as 10 cc. of blood from a single individual by heart puncture. Over 70 cc. of blood was obtained from an individual by rupturing the heart. The specific gravity of the blood was 1.0297, and, as the blood does not clot the corpuscles could easily be separated from the plasma, when the blood is left to stand. The plasma is pale yellow in colour.

B. Results.

1. *Hydrogen ion concentration.* The alkalinity of the blood in *Arca* was extraordinarily high in comparison with that of the other animals and had a remarkable buffer action. Although the alkalinity of the blood showed pH 7.75 (salt and protein error disregarded) in the undiluted state, it grew more and more alkaline by dilution, for instance, the alkalinity rose to pH 7.84 in 6 fold dilution and to 7.88

in 10 fold dilution. From the curves (Fig. 3) it is obvious that the rise of alkalinity reached the maximum at about the 11 fold dilution. By further dilution the alkalinity decreased, e.g. pH 7.78 in 51 fold dilution and pH 7.68 in 101 fold dilution.

TABLE XV.

Case No.	pH Degree of dilution						CO_2-gas		Unsaturated		Dimensions of animals			Tims (1925)		
	Un-diluted	6 fold	11 fold	21 fold	51 fold	101 fold	Content vol. %	Capacity vol. %	Portion	Percentage	Length (cm.)	Height (cm.)	Weight (gms.)	Month	Date	Hour
1	—	7.88	7.88	7.89	7.75	7.60	6.3	11.4	5.1	44.7	142	101	480	VIII	16	8.45 a.m.
2	—	7.80	7.80	7.78	7.78	7.50	5.6	9.2	3.6	39.1	142	105	685	VIII	16	11.30 a.m.
3	—	7.70	7.70	7.69	7.61	7.52	5.3	9.7	4.4	45.4	144	95	606	VIII	17	3.00 p.m.
4	—	7.83	7.85	7.80	7.75	7.68	5.4	10.1	4.7	46.5	123	84	367	VIII	23	9.30 a.m.
5	7.80	7.85	7.95	7.90	7.75	7.68	6.7	12.7	6.0	47.2	146	100	613	VIII	23	4.10 p.m.
6	7.70	7.83	7.85	7.90	7.75	7.68	7.9	14.6	6.7	45.9	142	101	558	VIII	23	4.40 p.m.
7	7.75	7.95	7.96	7.98	7.96	7.94	7.4	16.4	9.0	54.9	141	98	610	VIII	23	5.30 p.m.
8	7.73	7.86	7.90	7.88	7.76	7.68	6.5	12.9	6.4	49.6	137	95	520	VIII	24	9.20 a.m.
9	—	7.84	7.94	7.92	7.78	7.73	6.1	15.4	9.3	60.4	135	94	490	VIII	24	11.30 a.m.
10	7.75	7.84	7.90	7.91	7.80	7.68	6.9	12.9	6.0	46.5	136	95	507	VIII	24	3.30 p.m.
11	7.75	7.86	7.87	7.87	7.75	7.69	5.9	11.1	5.2	46.8	147	102	665	VIII	24	4.00 p.m.
12	7.75	7.85	7.89	7.89	7.80	7.75	5.4	12.4	7.0	56.5	143	102	650	VIII	24	4.30 p.m.
13	—	7.84	7.90	7.91	7.85	7.75	5.9	12.7	6.8	53.5	132	90	395	VIII	24	5.00 p.m.
14	—	7.82	7.86	7.85	7.77	7.60	6.6	12.3	5.7	46.3	125	84	377	VIII	24	5.30 p.m.
15	—	7.86	7.90	7.91	7.79	7.76	6.0	12.5	6.5	52.0	135	100	510	VIII	24	6.00 p.m.
Mean	7.75	7.84	7.88	7.87	7.78	7.68	6.3	12.4	6.2	49.0	—	—	—	—	—	—

The pH, CO_2- content and -capacity of the serum of *Arca*.

2. *CO_2-gas content and capacity.* According to the fifteen determinations referred to in the above table, the CO_2-gas content showed 5.3 to 7.9 vol. % with an average value of 6.3 vol. %, and the CO_2-gas capacity was 9.2 to 16.4 vol. % with an average value of 12.4 vol. %, indicating the difference of 6.1 vol. % when compared with the CO_2-gas content. The unsaturated portion of each determination was 3.6 vol. % as the minimum, 9.3 vol. % as the maximum, and 6.2 vol. % as an average. The unsaturated percentage was 39.1 vol. % as the minimum, 60.4 vol. % as the maximum, and 49.1 vol. % as an average.

III. DISCUSSION.

The animals employed for the present investigation consisted of 1 mammal, 5 teleosts, 1 selachia, 1 mollusca, and 1 holothuria. The mammal, the guinea pig, was observed in order to compare it with the cold blooded animals used. Among fishes, *Mustelus* came first and showed the maximum alkalinity of pH 7.72 in 16 to 21 fold dilution and the minimum value of pH 7.63 in 101 fold of dilution. *Cyprinus*, a fresh-water inhabitant, represents the second in order, its blood showing pH 7.63 in 6 fold dilution. *Pseudomonacanthus* was the third for its blood indicates the pH value 7.49 in 11 to 21 fold dilutions, and 7.47 in 101 fold dilution. The fourth was *Leuciscus*, which showed the pH value of 7.45 to 7.35 in dilutions of 6 to 101 fold. The fourth is *Seriola* in which the blood showed pH 7.40 in 6 fold dilution and 7.25 in 101 fold dilution. *Lateolabrax* and *Pagrosomus* interest us, inasmuch as both the forms showed the same pH value of 7.53 in 6 to 51 fold dilutions, while the former indicated pH 7.38 and the latter 7.33 in 101 fold dilution. Lastly, *Salmo* showed the lowest pH value of 7.27 to 7.29 in dilutions of 6 to 101 fold.

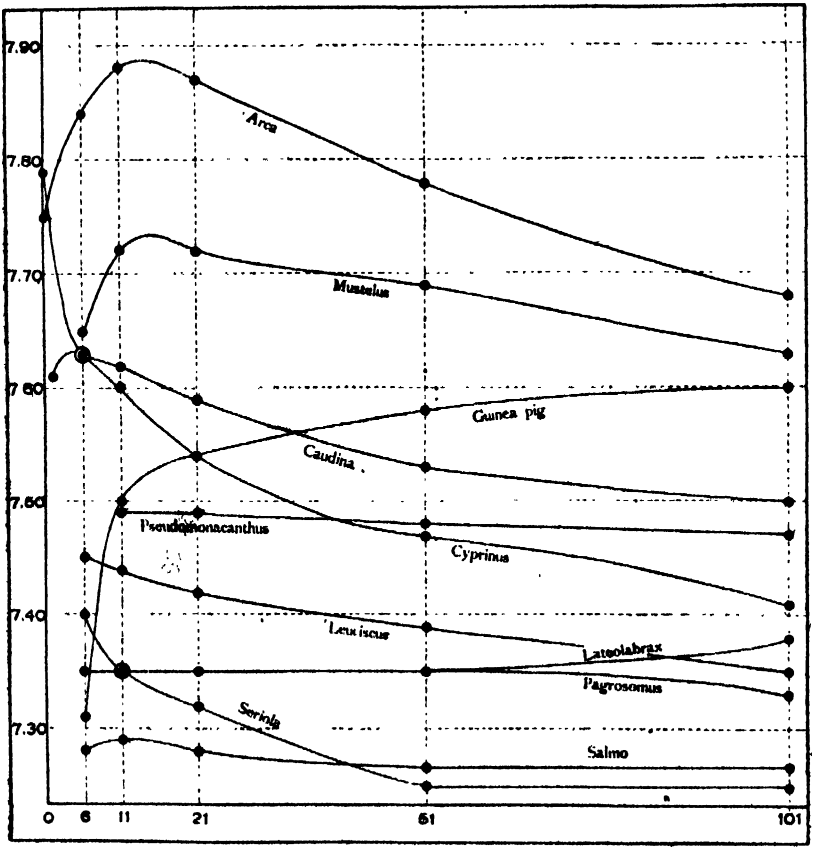
TABLE XVI.

No.	Species.	pH Degree of dilution 6 fold	11 fold	21 fold	51 fold	101 fold
1	*Mustelus manazo*	7.65	7.72	7.72	7.69	7.63
2	*Cyprinus carpio*	7.63	7.60	7.54	7.47	7.41
3	*Salmo iridius*	7.28	7.29	7.28	7.27	7.27
4	*Leuciscus hakuensis*	7.45	7.44	7.42	7.39	7.35
5	*Seriola aureovittata*	7.40	7.35	7.32	7.32	7.25
6	*Pseudomonacanthus modestus*	—	7.49	7.49	7.48	7.47
7	*Lateolabrax japonicus*	7.35	7.35	7.35	7.35	7.38
8	*Pagrosomus major*	7.35	7.35	7.35	7.35	7.33
9	*Caudina chilensis*	7.63	7.62	7.59	7.53	7.50
10	*Arca inflata*	7.84	7.88	7.87	7.78	7.68
11	*Guinea pig*	7.31	7.50	7.54	7.58	7.60

A comparison of the pH value in each species for the same dilution.

Bearing in mind the change of the pH value caused by dilution, we will note the fact that the alkalinity increases in low dilutions in *Musterus* and *Cyprinus*, though it decreases, on the contrary, in 15 fold or higher dilutions (Fig. 3). If one compares this connection with that of higher animals, for instance, the guinea pig, in which the alkalinity continually rises with increasing dilution, a remarkable distinction can be recognised. In the sea-fish the changes of alkalinity caused by dilution seems to be not so significant as in the animals referred to above. For instance, the alkalinity of the blood of *Pseudomonacanthus* showed just a faint decrease by the dilutions from 11 to 101 fold; in *Lateolabrax* and *Pagrosomus* it was just the same alkalinity at 6 to 51 fold dilutions, showing no change of the pH value at all, only in the cases over 51 fold dilution the *Lateolabrax* increases its blood alkalinity a little, while the *Pagrosomus* decreases it slightly. Among the sea-fishes, *Seriola* was the only example which showed a faint decrease of alkalinity at the beginning, while it showed no change at 50 to 101 fold dilutions.

FIG. 3.



Ordinate — pH value.
Abscissa — Degree of dilution (fold).

Comparing the mean pH value of 6 to 21 fold dilutions arrived at in different species of fishes, with horse plasma as determined by CULLEN (1922) with the same method, which gives the value pH 7.65 (20)° at 20 fold dilution, we find that *Cyprinus* comes nearest to the horse, and other sea-fishes in general give a much lower value, with the exception of *Mustelus*, which gives much higher values. According to HAWKINS (1923), who used the same method, the arterial and the venous blood of the rabbit showed pH 7.38 and pH 7.32 respectively, and the human venous blood indicated also the alkalinity of pH 7.32 in normal cases. All these values just cited approach the pH value of the sea-fishes, in particular that of *Lateolabrax Pagrosomus* and *Seriola*. Among the fishes *Seriola* in 51–101 fold and *Pagrosomus* in 101 fold dilution give lower alkalinity than that of the human venous blood (pH 7.31 by HASSELBALCH and 7.35 by MICHAELIS). With the exception of these three kinds of fishes just cited, all the animals observed showed a much higher alkalinity than in the case of human blood, especially the case of Selachia (*Mustelus*) in which the blood alkalinity was extraordinarily higher than in the case of Mammalia.

Investigation concerning the blood gas of fish has hitherto been very limited. As far as I am aware, JOLYET and REGNARD (1887) were the first who estimated the CO_2-gas in the blood obtained from the gill artery of the eel, and determined the quantity to be 33 vol. %. BAUMERT (1852) analyzed, on the other hand, the intestinal gas of *Cobitis fossilis*, which was believed to be equilibrated with the blood of the intestinal walls, and determined the CO_2-gas quantity to be 0.05–2.64 vol. %, suggesting that the CO_2-gas content of the blood must be the same as this. COLLIP (1920) determined the carbon dioxide content (using the blood equilibrated with the atmospheric air) and the capacity of the blood and coelomic fluid of marine fishes and invertebrates (using the blood equilibrated with the alveolar air). He stated that the carbon dioxide content of these fluids is in all cases very low as compared with the blood of mammals, nevertheless it is always higher than that of the sea water. As can be seen in my present investigation, the hydrogen ion concentration of the blood of marine forms is in most instances higher than that of the sea water, which was determined by other investigators, and, inspite of such a relation, the bicarbonate content of the former is much higher than that of the latter. COLLIP also became aware of this relation in his investigation and interpreted the lower hydrogen ion concentration by low bicarbonate and high hydrogen ion concentration by high bicarbonate content. This phenomenon just studied is due to the tension of the free carbon dioxide, which regulates the acid-base balance of the blood. Furthermore, he stated the possibility of the gill membrane's producing a steep pressure gradient of free carbon dioxide between the sea water and the blood. POWERS (1922) studied the relation between the oxygen absorbing power of fish blood and the hydrogen ion concentration of the environmental water, and suggested the possible significance of blood alkali, stating that the fish whose blood is best adapted to absorb oxygen at its low tension at the particular carbon dioxide pressure would survive longest, and this ability would perhaps depend upon the alkali reserve of the blood of the fish. Further more, he suggested that the variation in the ability to absorb oxygen at a low tension at a given pH of a species is dependant upon the alkali reserve of the blood of the individual fish. On the other hand, PACKARD (1905) showed that *Fundulus heteroclitus* injected with 5/16M. sodium carbonate are able to live longer in a tightly stopped flask of sea-water than others not so treated. If this statement is granted, the fish which has the largest alkali reserve should have the largest ability to absorb oxygen at low tension. From this stand point of view the CO_2-gas content of blood of a given species of fishes must be of such importance as to be worthy of notice. According to COLLIPS, the carbon dioxide content of the blood of the marine teleost is approximately 10 volume percent, while the same of the elasmobranchs is relatively very low (3.7 to 9.3 vols. %). The CO_2-gas content of fish blood, however, is doubtless the subject of much variation according to the species. For instance, the CO_2-gas content of the blood of *Cobitis*, found by BAUMERT, differs by far from that of the eel found by JOLYET and REGNARD. In the present investigation also a considerable difference due to the difference in species is found.

1) Carp (*Cyprinus*)	45.2 vol. %
2) Rainbow trout (*Salmo*)	24.6 „
3) Ugui (*Leuciscus*)	18.3 „
4) Umadarahagi (*Pseudomonacanthus*)	9.7 „
5) Hosizame (*Mustelus*)	9.1 „

6) Sironamako (*Caudina*)	8.5 vol. %
7) Akagai (*Arca*)	6.3 ,,

In *Cyprinus*, for instance, the blood has a very high content of 45.2 vol. %, i. e., just the same as that of the human arterial blood, while the *Pseudomonacanthus*, a sea fish, has a very low content of 9.7 vol. %, i. e., one-fifth of that of the human blood. In *Mustelus* the CO_2-gas content is still smaller, showing 9.1 vol. %. Beyond the limits of fishes, *Caudina* had a lesser value of 8.5 vol. %, and *Arca* showed a smaller value of 6.3 vol. %, which is not far from the CO_2-gas content of the sea-water tested by the same method.

In regard to *Caudina* and *Arca*, we found that the latter shows the highest pH value among all others thus far examined, including the *Guinea* pig and the fishes, and, similarly, that *Caudina* ranks high among all these mentioned above. Indeed *Arca* blood shows pH 7.88 in 11 fold dilution and pH 7.68 even in 101 fold dilution, and that of *Caudina* indicated a maximum pH value of 7.79 in the undiluted state, pH 7.63 in 6 fold dilution, and pH 7.50 in 101 fold dilution. Similar to the fish blood, *Arca* shows also an increase of alkalinity in low dilutions and a decrease in increasing dilutions. *Caudina* blood shows a higher blood alkalinity than *Arca* in regard to the undiluted plasma, this decreases rapidly by dilution showing a far lower alkalinity than *Arca* even in dilutions of over 6 fold.

In other details the reader is referred to the various tables given in the text. It seems premature to compare directly the vertebrate blood with the invertebrate without further data concerning morphological and physiological studies on the rest of the body. I therefore wish to reserve comparison between these two forms, invertebrata and vertebrata, for a future occassion.

IV. SUMMARY.

1. As far as my study goes, the blood of the aquatic animals employed in this experiment decreases in pH value by dilution. This is quite contrary to the case of Mammalia, in which the pH value increases continually by increasing the dilution, as ascertained by CULLEN (1922) and the present author as well. In *Arca*, *Mustelus*, and *Cyprinas*, however, an increase of the pH value due to dilution is noticeable at low dilutions only.

2. Among the animals employed in the present investigation *Arca* has the highest and *Seriola* has the lowest blood alkalinity. The order of these animals according to the pH value is as follows: *Arca, Mustelus, Caudina, Cyprinus, Pseudomonacanthus, Lateolabrax, Pagrosomus, Seriola.*

3. The increase of the pH value due to low dilution is most remarkable in *Arca; Mustelus* follows this and it is very faint in *Cyprinus*.

4. The blood of *Caudina* rapidly decreases its alkalinity by dilution and the rate of decrease is most prominent at dilutions of 0–6 fold.

5. According to the present investigation, the pH value of the blood of Teleost is very low when compared with that of *Mustelus*, and the increase of alkalinity by dilutlon in Selachia is more remarkable than in Teleost.

6. The pH value of the blood of the lower aquatic animals seems to be much higher than that of the higher animals, and probably this may have some phylogenetic meaning.

7. The CO_2-gas content of the blood of the fish much differs not only according to the species but also according to the physiological condition of the fish.

8. In *Arca* the CO_2-gas content is much less than that of fish and is nearly equal to that of sea-water examined by the same method.

BIBLIOGRAPHY.

1) HENDERSON, Y. 1925. Physiological regulation of the acid-base balance of the blood and some related functions. Physiol. Rev., Vol. V, No. 2, pp. 132–160.

2) BARCROFT, J. 1925. The respiratory function of the blood. pt. 1, pp. 88–102.

3) CULLEN, G. E. 1922. The colorimetric determination of the hydrogen ion concentration of blood plasma. Jour. Biol. Chem., Vol. LII, No. 2, p. 501.

4) MYERS, V. C. and etc. 1923. A micro colorimetric method of estimating the hydrogen ion concentration of the blood. Jour. Biol. Chem. Vol. LVII, pp. 209–216.

5) HAWKING, J. A. 1923. A micro method for the determination of the hydrogen ion concentration of the blood. Jour. Biol. Chem. Vol. LVII, No. 2, pp. 494–495.

6) HOLLO, J. u. WEISS, ST. 1924. Einfache Methode zur direkten Bestimmumg der Wasserstoffzahl des blutes mittels Indikatoren. Biochem. Zeitsch., Bd. 144, Heft 1–2, s, 87.

7) POWERS, E. B. 1922. The alkaline reserve of the blood of fish in relation to the environment. Amer. Jour. Physiol., Vol. LXI, pp. 380–383.

8) POWERS, E. B. 1922. The physiology of the respiration of fishes in relation to the hydrogen ion concentration of the medium. Jour. Gen. Physiol. Vol. IV, p. 305.

9) COLLIP, J. B. 1920. The alkali reserve of marine fish and invertebrates, Jour Biol. Chem., Vol. XLIV, p. 329.

10) PACKARD, W. H. 1905-1906. On the resistance to lack of oxygen and on a method of increasing this resistance. Amer. Jour. Physiol. Vol. XV, pp. 30-41.

11) KROGH, A. and LEITCH, I. 1919. The respiratory function of the blood in fishes. Jour. Physiol. Vol. LII, p. 288.

12) HAGGARD, H. W. and HENDERSON, Y. 1920. How oxygen deficiency lowers the blood alkali. Jour. Biol. Chem. Vol. XLIII, p. 15.

13) TRENDENBURG, P. 1912. Über die Sauerstofftension im blut von Seefischen. Zeitschr. f. Biol. Bd. LVII, s. 495-506.

On Two New Species of Mantispidae from Japan.

By

MASAYASU YAZAKI.

(With one plate)

I have stayed for the past ten years at Kagoshima, Kagoshima Prefecture. During that period I had chances from time to time to collect neuropterous insects of the family Mantispidae. In the collection thus far obtained there exist seven species which belong to the genus *Climaciella*. Five are referable to species hitherto known, while the remaining two seem to be new to science.

The following is the list of the species.

1. *Climaciella subfusca* NAKAHARA.
2. „ *habutsuella* OKAMOTO.
3. „ *4-tuberculata* (WESTWOOD).
4. „ *miyakei* OKAMOTO.
5. „ *magna* (MIYAKE).
6. „ *satsumensis*, n. sp.
7. „ *tanegashimensis*, n. sp.

In the present paper I propose to give the descriptions of the new species and to append tables which show several characteristic features of all the Japanese species of the genus.

Before entering the subject, I wish to express my best thanks to Prof. HOZAWA for his kind review of the manuscript.

Climaciella satsumensis, n. sp.

Male.—Head, black, with frons orange yellow; maxillary and labial palpi, ochraceous yellow; eyes, black; antenna, ochraceous with 23 segments, basal segment enlarged, twice as long as broad, the terminal segment spindle-shaped.

Prothorax, dark brown with numerous fine transverse furrows, the anterior dilated portion orange yellow, with anterior margin dark fulvous.

Meso- and metathorax, black, with yellow scutella.

Wings, hyaline with fuscous veins, costal areas with brown band, macrotrichia and microtrichia, dark brown and fairly densely disposed. Fore-wing with three veins from first radial cell and two veins from second radial cell; hind-wing with three veins from both the first and the second radial cells; anal veins, simple, not crotched.

Legs, ochraceous yellow, except the coxae, which are dark brown. Middle portion of the raptorial femora of fore-legs, dark brown, their inner side being darker than the outer. In the mid- and hind-legs, two spines at the extremity of the tibiae brown; each of them from the 1st to the 4th tarsal segments is provided with two terminal black spines and 7-8 ventral setae of the same colour and is beset with long, dark brown hairs. The tip of the tarsal claw is split into three teeth, the middle of which is always longer than the others.

In the abdomen the anterior half of each dorsal segment is brown, but darker posteriorly; the ventral segments are equally sepia coloured. A large chitinous plate projects on the last dorsal abdominal segment and is directed posteriorly. A long needle-like projection also occurs at the base of the plate.

Measurements:

Length of body	11.0 mm.
Width of head	2.0
Length of head	0.8
Length of antenna	2.0
Length of prothorax	3.2
Length of fore-wing	11.8
Width of fore-wing	3.0
Length of fore-femur	4.0
Expanse of wings	24.9

Locality. Ibusuki, Kagoshima Prefecture. A single male was taken by myself on September 5, 1925.

Remarks. This mantispid resembles *C. 4-tuberculata*, from which it is easily separated by its smaller size, differences in colour and markings, venation, and the structure of the posterior abdomen. The number of teeth on the tarsal claw is the most characteristic feature by which the present species may be easily distinguished from most of the other species. In *Climaciella* it is not common that the tarsal claw has three teeth, and such case is hitherto known only in *C. magna* (MIYAKE).

Climaciella tanegashimensis, n. sp.

Female. — Head, orange yellow, with three black transverse lines between the antennae, on the clypeus and on the occiput respectively; trophi, orange yellow; mandibles, black; antennae, black; along the median line of the head there is a deep groove which arises between the antennae and terminates at the occiput. Antennae, dark brown with 30 segments, the basal segment is conspicuously larger than the second, the last segment is spindle-shaped, with many basiconic sensillae.

Prothorax, pitch-dark in colour with some long transverse impressions, the anterior dilated portion, orange yellow and with its anterior margin pitch-dark. On both the anterior and the posterior portions there occur two warts.

Meso- and metathorax, black, with its dorsal median line pale yellow; near the hind part of the scutum there occurs an oblong projection, the posterior part of the scutellum bears a small process (a tubercular process on the mesothrax, a spindle-like process on the metathorax); in the middle part of the scutum, moreover, there is a deep concavity with a yellowish margin.

Wings, hyaline; veins, brownish yellow, costal area with a pale brownish broad band, both microtrichia and macrotrichia dark brown; the microtrichia on the wing-membrane are somewhat sparsely disposed. Fore-wings with three veins from both the first and the second radial cells; hind-wings with four veins from the first radial cell and three veins from the second cell; anal veins, simple, not crotched.

Fore-legs, dark brown, with trochanter black; femora and tarsi with yellow and black hairs mixed; mid- and hind-legs, dark brown, with pleural suture black. Tip of each claw splits into three teeth, the middle of which is longer than the others.

Dorsal surface of the abdomen, yellowish brown, but with the 1st, 2nd, 4th, and 6th segments black; ventral surface of the same, black, with the 6th segment yellow. On the middle part of the 2nd dorsal segment there occurs a tubercular process. Extremity of the abdomen bears long dark brown hairs thickly disposed.

Measurements:

Length of body	15.0 mm.
Width of head	2.8
Length of head	1.0
Length of antenna	2.8
Length of prothorax	4.0
Length of fore-wing	15.0
Width of fore-wing	4.8
Length of fore-femur	5.0
Expanse of wings	31.5

Locality. Tanegashima, Kagoshima Prefecture. Two females were taken by myself on September 8, 1925.

Remarks. The species which stands in the nearest relationship to this new species is *C. habutsuella* OKAMOTO. They differ from each other in the structure of the wing and in the form of the mesothorax, as well as in the form of the tarsal claw.

Here I appended two tables showing the most important characters of the five hitherto known Japanese species and of the present two new species of the genus *Climaciella*.

	Length of body	Number of segments of antenna	Number of teeth of tarsal claw
C. subfusca	11.5 mm.	—	5
C. 4-tuberculata	12.0	27	4
C. habutsuella	13.0–14.0	28	5
C. miyakei	20.0–22.0	31	5
C. magna	27.0	40	3
C. satsumensis	11.0	23	3
C. tanegashimensis	15.0	30	3

	Fore-wing			Hind-wing	
	Veins from $1R_1$	Veins from $2R_1$	Veins from costal cell	Veins from $1R_1$	Veins from $2R_1$
C. subfusca	3	3	1	3	2
C. 4-tuberculata	4	3	8–9	4	3
C. habutsuella	4	3	8–9	4	3
C. miyakei	3–5	3–4	8–9	3–5	3–4
C. magna	5–6	5–6	14–15	5	5–6
C. satsumensis	3	2	11	3	3
C. tanegashimensis	3	3	10	4	3

REFERENCES.

1) Banks, N. 1913. Synopsis and Descriptions of Exotic Neuroptera. Trans. Am. Ent. Soc. Washington. Vol. XXXIX. pp. 205–211.

2) Brauer, F. 1867. Zur Kenntniss der Mantispiden.

3) Enderlein, G. 1910. Klassifikation der Mantispiden nach dem Material des Stettiner. Zool. Museums. Stettin. entom. Zeitg. Bd. LXXI. pp. 341–379.

4) Esben-Petersen, P. 1917. Neue und wenig bekannte Mantispiden. Arkiv. f. Zool. Bd. XI. pp. 1–15.

5) Kuwayama, S. 1925. Notes on the Japanese Mantispidae with Special Reference to the Morphological Characters. Jour. Coll. Agr. Hokkaido Imp. Univ. Sapporo, Vol. XV. pp. 237–267.

6) Miyake, T. 1910. The Mantispidae of Japan. Jour. Coll. Agr. Imp. Univ. Tokio, Vol. II. pp. 213–221.

7) Nakahara, W. 1912. Studies on the Mantispidae of Japan. (In Japanese) Zool. Mag. Vol. XXIV. pp. 558–566.

8) Nakahara, W. 1913. A Revision of the Mantispidae of Japan. Ann. Zool. Japonenses Vol. VII. pp. 229–237.

9) Okamoto, H. 1910. The Mantispidae of Japan. (In Japanese) Zool. Mag. Vol. XXII. pp. 533–544.

10) Okamoto, H. 1911. Beitrag zur Mantispiden-Fauna Japan. Zool. Anz. Bd. XXXVII. pp. 293–302.

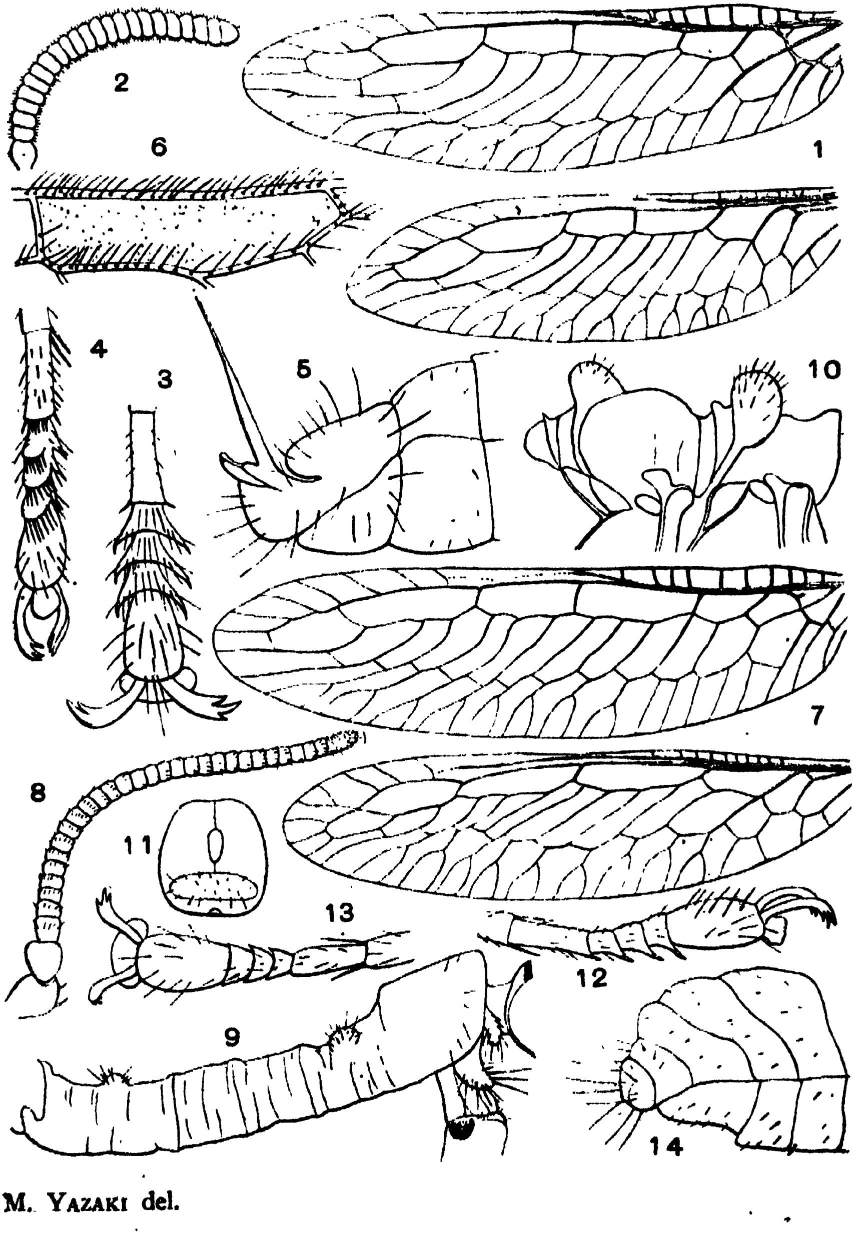
11) Stitz, H. 1913. Mantispiden der Sammlung des Berliner Museums. Mitt. Zool. Mus. Berlin Bd. VII. pp. 1–49.

12) Walker, F. 1853. Catalogue of the specimens of Neuropterous insects in the collection of the British Museum, Pt. II. p. 213.

EXPLANATION OF PLATE.

Fig. 1. *Climaciella satsumensis*, n. sp. Fore- and hind-wings.
Fig. 2. Ditto. Antenna.
Fig. 3. Ditto. Dorsal view of hind tarsus.
Fig. 4. Ditto. Lateral view of hind tarsus.
Fig. 5. Ditto. Lateral view of terminal segments of abdomen. Showing the remarkable chitinous plate and conspicuous spine.
Fig. 6. *Climaciella satsumensis*, n. sp. Structures of 1R of fore-wing. Showing the macrotrichia and microtrichia.
Fig. 7. *Climaciella tanegashimensis*, n. sp. Fore- and hind-wings.
Fig. 8. Ditto. Antenna.
Fig. 9. Ditto. Lateral view of prothorax.
Fig. 10. Ditto. Lateral view of meso- and metathorax.
Fig. 11. Ditto. Dorsal aspect of mesothorax with a deep concavity.
Fig. 12. Ditto. Lateral view of hind tarsus.
Fig. 13. Ditto. Dorsal view of hind tarsus.
Fig. 14. Ditto. Lateral aspect of terminal segments of abdomen.

M. YAZAKI. Two new species of Mantispidae.



M. YAZAKI del.

Sur la Croissance Normale du *Paramecium caudatum*.

De

Fukuichiro Mizuno

Lorsque Leeuwenhoek découvrit en 1675 avec l'espèce Vorticella une autre espèce de protozoaires ce fut sans doute la premiere découverte de l'espèce *Paramecium*. Par la suite le *Paramecium caudatum* et le *Paramecium aurelia* ayant une forme plus grosse et se trouvant un peu partout furent les plus étudiés des protozoaires.

Il s'ensuit que la croissance du protozoaire a été jusqu'ici étudiée profondément, mais on avait porté surtout l'attention sur sa multiplication, sans étudier pour ainsi dire sa croissance. Cependant ce dernier point a été étudié par Pearl (1907), Jennings (1908), Rautman (1909), Popoff (1909), Estabrook (1910), Hargitt et Fray (1917), Phillips (1922), et Schmalhausen (1925).

Parmi ces derniers Jennings et Schmalhausen seuls se sont occupés de cette croissance normale. Jennings a procédé par la dessiccation du *Paramecium* et en a mesuré la largeur et la longueur des cellules; tandis que Schmalhausen en a étudié les cellules vivantes. Je voulus moiaussi faire cette étude sur des *Paramecium* vivants, mais je ne pus réussir à en prendre les mesures. Alors ayant recouru à la dessiccation je pus mesurer la longueur, la largeur et l'aire des- dits protozoaires. Au moyen de ces trois dimensions je pus étudier la croissance normale des *Paramecia* et cette thèse est le résultat de cette étude.

MATIÈRE ET MÉTHODE

Pour cette étude je me suis servi de *Paramecia* recueillis dans un fossé (égout) qui se trouve au près de l'université. J'en séparai un seul que j'élevai dans une infusion de foin et le multipliai. Grâce à cette multiplication je reconnus sans erreur l'espèce *Paramecium caudatum* et ce fut la donnée de mon étude.

Pour faire l'infusion j'employai 6 grs. de foin 500 c.c. d'eau distillée que je fis bouillir une demi-heure, et laissai reposer toute la journée avant de m'en servir. Chaque semaine je renouvelai cette infusion pour multiplier et pour conserver les protozoaires. Pour ce faire je prenais de 20 à 30 cc. d'eau de l'infusion précédente. Pour obtenir la croissance, j'employai comme aliment L'Extrait de Viande Liebig employé déjà par les expérimentateurs précédents, en solution de 0.025%. Dans une expérience préliminaire je compris que ce pourcentage était la plus efficace pour la multiplication des protozoaires. Cette solution de Liebig fut introduite dans l'éprouvette bien bouchée d'ouate et stérilisée à l'autoclave. Le jour précédent, choisissant la bactérie, j'introduisis le *Bacillus fluorescens liquifaciens* et chauffai l'éprouvette entre 27° et 32°C.

Ph. de la culture medium au premier jour était à 6.2-6.8 et le jour suivant, par le fait des bactéries Ph. arrivait à 5 environ et peu à peu l'alcali vint à apparaître, comme du reste cela se produisait dans l'infusion de foin, ce que Bodine avait déjà vérifié en 1921. Dans l'infusion de foin le changement de l'acide en alcali est à son plus haut degré le 3ème et le 4ème jours et c'est à ce moment que le *Paramecium* se divisat le plus.

Pour contrôler la température, j'employai l'étuve. A la témperature variant de 24° à 26°C. le *Paramecium* se divisa en deux au bout de 8 à 9 heures. C'est le même résultat qu'obtint Popoff (1909). Je voulus vérifier s'il n'y avait pas de différence de multiplication à l'étuve noire, ou à la lumière, et je constatai comme Maupas (1888) et Woodruff (1905) l'avaient déjà fait que cette différence n'existait pas. Donc dans les ténèbres ou à la lumière il n'y avait pas de différence comme le prétendit Dembowski (1922).

Après le troisième ou le quatrième jour de l'inoculation du medium dans l'infusion de foin, j'en puisai une petite partie et en l'examinant au microscope à dissection, je pus saisir sans difficulté le moment de la division du *Paramecium*. J'en saisis un avec la pipette effilée et je l'insérai dans le medium Liebig chauffé préalablement à 25°C. La division se faisait parfois aussitôt, d'autrefois elle s'opérait au bout de 30 minutes environ. Après avoir noté le temps de la divison, je placai chaque *Paramecium* sur une lame à concavité, dans laquelle se trouvaient à peu près 0.015 c.c. de medium Liebig. Ensuite j'enfermai cette lame chacune dans une boite de Patri, et pour empêcher l'évaporation de medium je plaçai ces boites dans une étuve. Après un temps déterminé, je saisis le *Paramecium* avec la pipette effilée pour le placer sur une soucoupe de verre. Après avoir epuisé le plus possible le medium liquide, je versai beaucoup de " Worcestor's fluid " pour tuer le dit *Paramecium*. Ce fluide contient une solution saturée de sublimé corrosif avec 10% de formaline. J'essyai la solution employée par BOUIN et SCHAUDIN, mais ce " worcestor's fluid " a été le plue efficace. Puis je mis dans la glycerine ce *Paramecium* mort pour le conserver humide et transparent. Ensuite je le plaçai sur une lame de verre, et pour ne pas l'écraser avec la lamelle supérieure. J'eus soin de mettre entre les deux un poil de marmotte. Je grossis mon échantillon 500 fois au microscope, je pris son esquisse avec l'appareil à dessiner d'Abbe, et je mesurai au planimètre sa surface non sans avoir noté sa longueur et sa largeur. Le résultat de ces mesures est consigné d'après la méthode statistique inserée dans ce travail.

RÉSULTAT

Avec les Tables (I, II, III, IV.) et avec la carte échelle (Fig. 1.) ci-joints on comprendra facilement mon assertion. A la fin de cette étude j'ai inséré des tables de corrélation qui la rendront plus évidente.

TABLE I.

Temps d' observation	Nombre d'individus	Longueur			Differences de variation (μ)
		Moyen (μ)	Deviation type (μ)	Coefficient de variation	
Immédiatement après la scission	80	125.687± .637	8.441±.450	6.716±.357	106-142
5 minutes après	50	142.000± .751	7.874±.531	5.545±.374	128-155
15 minutes après	51	155.333± .824	8.726±.583	5.618±.376	187-176
30 minutes après	51	158.667± .665	7.048±.460	4.442±.304	187-179
45 minutes après	50	160.300± .767	8.038±.542	5.014±.340	143-181
1 heures après	65	162.846± .717	8.574±.507	5.265±.312	141-180
2 heures après	75	164.133± .739	9.493±.523	5.787±.557	144-184
4 heures après	99	167.051± .920	13.579±.651	8.129±.451	140-196
6 heures après	79	171.620±1.013	13.354±.717	7.781±.733	142-201
8 heures après	65	178.385±1.173	14.022±.829	8.087±.924	146-204

Immédiatement après la séparation en deux du *Paramecium*, la longueur de chacun d'eux était de 125.687μ., trente minutes après elle augmentait de 32.980μ. et après 30 minutes encore elle augmentait de nouveau de 4.179μ. et ensuite elle augmenta par heure de 1.287μ, 2.918μ, 4.561μ, et 2.765μ. On voit ainsi que pendant la première demi-heure elle augmente considérablement, dans la seconde demi-heure l'augmentation est moindre, puis elle grossit ensuite très lentement. Ce résultat est donc à peu près équivalent à celui obtenu par JENNINGS (1909) et SCHMALHAUSEN (1925). Parmi les individus, celui de la plus grande longueur arrivait presque au double de celui de la plus petite. La deviation type parait augmenter peu à peu, ce doit être d'après la condition des individus étudiés.

Quant à la largeur, la Tabble II. montre un résultat très intéressant.

TABLE II.

Temps d' observation	Nombre d'individus	Largeur			Differences de variation (μ)
		Moyen (μ)	Deviation type (μ)	Coefficient de variation	
Immédiatement après la scission	80	57.375± .666	8.830±.471	15.391± .834	41- 81
5 minutes après	50	41.500± .507	5.315±.358	12.807± .936	32- 53
15 minutes après	51	42.784± .722	7.642±.510	17.862± .230	31- 59
30 minutes après	51	42.686± .545	5.775±.386	13.540± .920	33- 56
45 minutes après	50	46.600± .547	5.600±.387	12.297± .842	33- 61
1 heures après	65	50.077± .710	8.489±.512	16.954±1.273	38- 71
2 heures après	75	54.800± .555	7.129±.393	13.009± .767	39- 75
4 heures après	99	60.687± .579	8.545±.410	14.081±2.175	44- 89
6 heures après	79	67.056± .779	10.264±.551	12.992±1.201	50- 91
8 heures après	65	72.692±1.144	13.684±.809	18.768±1.188	53-102

Aussitôt la séparation accomplie, la largeur qui était de 57.375μ. au bout de cinq minutes se trouvait réduite à 41.500μ. c'est-à-dire elle était diminuée de 15.875μ. et pour reprendre sa première largeur il fallait de 2 à 4 heures. La deviation type pour la largeur était selon

TABLE III.

Temps d' observation	L'index moyen $\left(\frac{\text{largeur}}{\text{longueur}}\right)$	Coefficient du correlation entre longueur et largeur	Coefficient du correlation entre la longueur et l'aire
Immédiatement après la scission	0.457	-0.128±.074	0.250±.071
5 minutes après	0.292	0.538±.068	0.352±.084
15 minutes après	0.275	0.343±.083	0.181±.091
30 minutes après	0.269	0.105±.093	0.408±.079
45 minutes après	0.298	0.402±.060	0.188±.092
1 heures après	0.308	-0.177±.101	0.135±.082
2 heures après	0.334	0.133±.076	0.585±.051
4 heures après	0.363	-0.380±.049	0.386±.068
6 heures après	0.391	-0.166±.085	0.298±.009
8 heures après	0.419	0.139±.010	0.656±.077

le temps la même que la longueur. JENNINGS (1908) avait constaté le même changement dans la largeur. Le plus large individu était trois fois plus large que le plus petit.

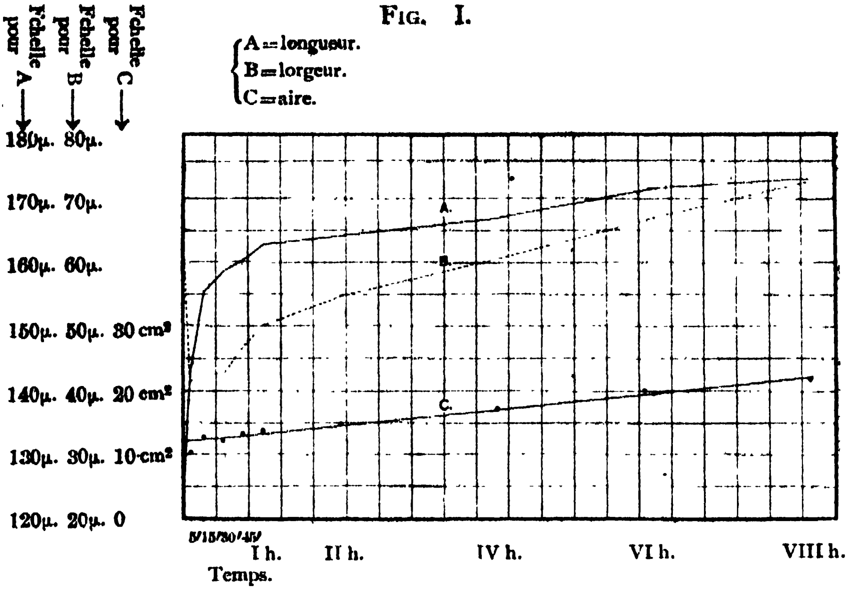
L'index moyen a donc tendance à changer avec la largeur. Aussitôt après la séparation du corpuscule la largeur augmentant et la longueur diminuant, le coefficient de corrélation cause tout naturellement un négatif. Mais au bout de cinq minutes, malgré la tendance apparente à la diminution de la largeur, par le fait de l'augmentation en longueur, la majorité des échantillons montre au contraire une tendance a l'augmentation de cette largeur et alors le résultat de corrélation est possitif. Ainsi dans la table de corrélation (p. 375, II) nous trouvons que parmi les 50 individus étudiés dans cette table a peu près la moitié c. a. d. 23 individus trouvent à 37μ. et le reste est distribué ça et là audessus de 37μ. Le minimum de largeur devait donc exister avant les cinq minutes qui suivent la scission. Au bout des cinq minutes la largeur avait dû se rétablir, pour annihiler ainsi ce négatif.

TABLE IV.

Temps d' observation	Nombre d'individus	Aire × 500			Differences de variation (cm^2.)
		Moyen (cm^2.)	Deviation type (cm^2.)	Coefficient de variation	
Immédiatement après la scission	80	12.200 ± .079	1.044 ± .056	8.559 ± .465	7.9–19.3
5 minutes après	50	10.460 ± .212	2.220 ± .150	21.223 ± 1.432	7.9–15.9
15 minutes après	51	12.294 ± .264	2.793 ± .187	22.721 ± 1.517	9.6–19.9
30 minutes après	51	12.235 ± .170	1.800 ± .120	14.711 ± .983	8.8–16.8
45 minutes après	50	13.460 ± .202	2.140 ± .143	15.896 ± 1.062	10.0–19.7
1 heures après	65	13.584 ± .176	2.099 ± .124	15.452 ± .914	10.5–17.2
2 heures après	75	14.920 ± .194	2.497 ± .138	16.735 ± .922	11.6–22.3
4 heures après	99	17.455 ± .151	2.231 ± .107	12.779 ± .613	13.4–24.2
6 heures après	79	19.734 ± .262	3.448 ± .185	17.474 ± .938	14.6–30.7
8 heures après	65	21.846 ± .346	4.135 ± .245	18.929 ± 1.120	15.4–33.2

Quant à l'aire c'est à peu près le même résultat que pour la largeur. Mais on n'y voit pas un aussi grand changement que dans la longueur ou dans la largeur. Après la scission cette aire diminue, mais pour se rétablir aussitôt. A ce moment la largeur étant plus forte le corpuscule est arrondi mais peu après la largeur se retrécissant, la surface diminue. Il s'agit uniquement de l'aire car le volume ne doit pas changer. Le maximum d'aire d'un individu est 4 fois plus grand que son minimum. La courbe de la croissance au point de vue de l'aire peut donc être considérée comme une ligne directe.

Fig. I.



CONSIDÉRATION GÉNÉRALE

Le phénomène de croissance du Métazoaire a été longuement étudié. En général la croissance organique signifie la croissance du volume ; le volume grossissant la masse doit grossir en même temps. C'est donc au moyen du poids qu'on a étudié cette croissance. La courbe de croissance est également indiquée par le poids. Mais pour les protozoaires, le corpuscule étant infime on ne peut le peser. Aussi leur croissance n'a été étudiée qu'extérieurement c'est-à-dire par la longueur et par la largeur (Jennings et Schumalhausen). Également on a mesuré leur volume d'après ces deux dimensions (Popoff). Mais comme le volume reste inconnu, on ne peut obtenir une juste notion de cette croissance par l'étude de la longueur et de la largeur. Donc bien qu'on ait pu faire le calcul de ce volume, ce n'est qu'un calcul approximatif. Mais ayant étudié cette croissance par l'aire, bien que ce n'ait pas été l'idéal encore, on peut vu les circonstances, le considérer comme le meilleur résultat obtenu jusqu'ici.

En joignant à l'étude de la longueur et de la largeur, celle de l'aire, on obtient un résultat très different. Car en étudiant la longueur seulement on constate qu'elle augmente beaucoup après la scission, et pour la largeur on constate son retrécissement, mais l'étude de l'aire indique que le changement est peu considérable. Ce changement doit être seulement à un manque d'étude par l'aire, car pour le volume real la raison indique qu'il n'y a pas de changement. Au bout de cinq minutes après la scission le corpuscule s'allongea pour prendre sa forme réguliere, mais sa croissance n'est pas réelle comme l'indiquerait sa longueur. Un pareil changement de forme et de dimensions a été profondément étudié par Child (1915), mais le cas du *Paramecium* semble être le cas le plus simple de reprise de forme.

En voyant la courbe de croissance de l'aire, on remarque comme je l'ai dit précédemment que la courbe de croissance du corpuscule est une courbe linéaire. En 1911 Hatai a expliqué mathématiquement toute courbe de croissance et il dit :. " An organism during growth tends to form the greatest amount of mass with least of growing capacity." En constatant que la courbe de croissance du *Paramecium* est une courbe linéaire on obtient une preuve typique de cette définition. La courbe de croissance du métazoaire si comparée à celle du *Paramecium*, il est évident que cette dernière est plus primitive.

RESUMÉ

1. D'après les études faites ci-dessus la croissance du *Paramecium caudatum* immédiatement après la scission apparait très grande selon la longueur acquise, mais en même temps sa largeur diminuant, l'aire indique que cette croissance est presque nulle. Il faut donc y voir seulement un changement de forme. Il enrésulte qu' en général la dimension du *Paramecium* ne doit pas être jugée seulement d'après sa longueur et sa largeur, mais la dimension de l'aire me semble être plus satisfaisante.
2. Etudiée par la méthode de l'aire la croissance du *Paramecium caudatum* est pratiquement une courbe linéaire, et, parmi les courbes de croissance, doit être considerée comme la plus primitive.

Je dois la plus profonde reconnaissance à Mr. le professeur S. Hatai pour les conseils précieux qu'il m'a donnés et les remarques si obligeantes qu'il a daigné me faire pour cette etude.

REFERENCES

1) BODINE, J. R. 1921. Hydrogen-ion concentration of protozoan cultures. Biol. Bull. Vol. 41, pp. 73-77.

2) CHILD, C. M. 1915. Senescence and Rejuvenescence. The Univ. of Chicago Press.

3) DEMBOWSKI, J. 1922. Sur l'infuluence de la lumière sur la rapidite des divisions chez Paramecium caudatum. Trav. Lab. Gen. de L'Inst. M. Nencki, I. (Je ne vis pas l'orginal).

4) ESTABROOK, A. H. 1910. Effect of chemicals on growth in Paramecium. Jour. Exp. Zool., Vol. 8. pp. 489–534.

5) HARGITT, G. T. and FRAY, W. W. 1917. The growth of Paramecium in pure culture of bacteria. Jonr. Exp. Zool. Vol. 22. pp. 421–455.

6) HATAI, S. 1911. An interpritation of growth curves from a dynamical standpoint. Anat. rec. Vol. 5. No. 8 pp. 373–382.

7) JENNINGS, H. S. 1908. Heredity, variation and evolution in protozoa. II. Heredity and variation of size and form in Paramecium, with studies of growth, enviromental action and seclection. Proceed. of Amer. Physiol. soc. Vol. 47. No. 190.

8) MAUPAS, E. 1888. Recherches expérimentales sur la multiplication des Infusoires cilies. Arch. de Zool. Exp. et Gen., Ser. 2, Tom. 6. pp. 165–277.

9) PEARL, R. 1907. A biometrical study of conjugation in Paramecium. Biomtrika, Vol. V. No. 3.

10) PHILLIPS, R. L. 1922. The growth of Paramecium in infusion of known bacterial contents. Jour. Exp. of Zool. Vol. 36. pp. 135–183.

11) POPOFF, M. 1909. Experimentelle Zellstudien. II. Über die Zellgrosse, ihre Fixierung und Vererbung. Arch. f. Zellforsch. B. III.

12) RAUTMANN, H. 1909. Der Einflus der Temp. auf das Grossenverhaltnis des Protoplasma-körpers zum Kern. Experimentelle Untersuchungen an Paramecium caudatum. Arch. f. Zellforsch. B. III.

13) SCHMALHAUSEN, I. 1925. Studien über Wachstum und Differenzierung. I. Die Individuelle Wachstumskurve von Paramecium caudatum. Arch. f. Entwmech. B. 105. Heft 4.

14) WOODRUFF, L. L. 1905. An experimental study on the life-history of hypotrichous Infusoria. Jour. Exper. Zool., Vol. 2. pp. 585–632.

Tableau de la Correlation entre Longueur et Largeur.

largeur longueur	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	
105–109				2	2	1				5
110–114	1	1			1		1			4
115–119			2	3	4					9
120–124		1	3	5	2	1	2			14
125–129	1	3	2	4	6		2			18
130–134	3	3	3		3	4	1		1	18
135–139	1	4	1	1	2	1	1			11
140–144			1							1
	6	12	12	15	20	7	7	0	1	80

I. Immediatement après la scission.

largeur longueur	30–34	35–39	40–44	45–49	50–54	
125–129		2				2
130–134		9	1			10
135–139		4		1		5
140–144	1	5	3	4	2	15
145–149		3	2	1	1	7
150–154			5	2	2	9
155–156				2		2
	1	23	11	10	5	50

II. 5 minutes après.

Tableau de la Correlation entre Longueur et Largeur.

largeur / longueur	30–34	35–39	40–44	45–49	50–54	55–59	
135-139		1	1	1			3
140-144	1	2				1	4
145-149	2	3					5
150-154	2	2	1	1		1	7
155-159		7	2	3	3	2	17
160-164		4	2	2	1		9
165-169		1			2	1	4
170-174						1	1
175-179				1			1
	5	20	6	8	6	6	51

III. 15 minutes après.

largeur / longueur	30–34	35–39	40–44	45–49	50–54	55–59	
135-139				1			1
140-144		1	1				2
145-149	1						1
150-154		3	1	2	1		7
155-159		6	4	2	3	1	16
160-164		7	3	6	1		17
165-169		2	2	1			5
170-174					1		1
175-179				1			1
	1	19	11	13	6	1	51

IV. 30 minutes après.

Tableau de la Correlation entre Longueur et Largeur.

largeur / longueur	30-34	35-39	40-44	45-49	50-54	55-59	60-64	
140-144		1		1				2
145-149								0
150-154	1	1	2	2	1			7
155-159		2	2	8	2		1	15
160-164		1	4	4	2	1		12
165-169			1	4	3	1		9
170-174			1	1				2
175-179					2			2
180-184						1		1
	1	5	10	20	10	3	1	50

V. 45 minutes après.

largeur / longueur	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	
140-144						2			2
145-149			1	1					2
150-154		2	1	1		1	1		6
155-159		3	5	2	1				11
160-164	1	6	3	1	1	3	1	1	17
165-169	1	5	1	2	2	1	1		13
170-174	1	1	3	2	1	1			9
175-179	1	1		1					3
180-184					1	1			2
	4	18	14	10	6	9	3	1	65

VI. 1 heure après.

Tableau de la Correlation entre Longueur et Largeur.

longueur \ largeur	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	
140–144				1					1
145–149	1		1	1	2				5
150–154		1	2	2	1	1			7
155–159		1	1	2	2	2			8
160–164		1	1	3	7		2	1	15
165–169			1	7	3	2	1	1	15
170–174		1	3	2		3			9
175–179				2	2	2	1	1	8
180–184				4					4
	1	4	9	24	18	10	4	3	75

VII. 2 heures après.

Tableau de la Correlation entre Longueur et Larguer.

largeur / longueur	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	
140–144				1	1	1	2				5
145–149				3	1	2					6
150–154				2	3	3	2				10
155–159		1			1	2	2	1			7
160–164		2	1	4	3	3	2	2			17
165–169	1		4	1	3	2				1	12
170–174		2	2	3	1	1	1		1		11
175–179		1	2	3	1	3					10
180–184			4	4	2		1				11
185–189		1		1	2						4
190–194		1	2	1	1						5
195–199					1						1
	1	8	15	23	20	17	10	3	1	1	99

VIII. 4 heures après.

Tableau de la Correlation entre Longueur et Largeur.

largeur / longueur	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	
140–144				2						2
145–149	1	1				2				4
150–154				1	1					2
155–159			2	1	3			1		7
160–164			1	2	1		1			5
165–169		2	3	2	3	2	1		2	15
170–174	1	1	4		5					11
175–179	5	1		1		1		2		10
180–184	2	3	1	1		1	3	1		12
185–189		1	2							3
190–194		1	2		1			1		5
195–199		1								1
200–204		1	1							2
	9	12	16	10	14	6	5	5	2	79

IX. 6 heures après.

Tableau de la Correlation entre Longueur et Largeur.

largeur / longueur	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–99	100–104	
145–149				2								2
150–154		1		1	1	1						4
155–159			1		1	1	1					4
160–164		1		2			1					4
165–169	1	1	3			1	2	1				9
170–174	1	2		1	4	1	1		2	1		13
175–179	1	1				2		1	2			7
180–184		2	5				1		1			9
185–189		1		1		1	1		1	2	1	8
190–194			1								1	2
195–199			1			1						2
200–204			1									1
	3	9	12	7	6	8	7	2	6	3	2	65

X. 8 heures après.

Report of the Biological Survey of Mutsu Bay.*

3. Notes on the Protozoan Fauna of Mutsu Bay.

I. Peridiniales.

By

TOHRU HIDÉMITI ABÉ.

(Zoological Institute, Faculty of Science, Tokyo Imperial University,
Tokyo, Japan.)

The investigation of the plankton of Mutsu-Bay carried on last August at the Asamushi Marine Biological Station has brought to light a rich protozoan fauna in which the Dinoflagellates are abundant. The material, as will be surmised from the conditions of the oceanic currents in the Tsugaru Strait, is comprised partly of a number of warm temperate water species and partly of those of cold temperate seas.

The present paper includes only the majority of the Peridiniales observed during my sojourn at the station last August. The rest of them and of other classes will be given in later papers. Not only the new forms which came to light in my investigations, but also the other species which have already been reported from warm and cold temperate seas in other parts of the world are figured, and some of them are fully re-described here. The difficulty in getting adequate literatures in this country forced me, to my great regret, to leave some, which seem to me recommendable, unidentified. The references under each species in this paper are those for which I have been able to see some figures concerning the species even though the original papers for the species whether I could or could not get. Of late years several different methods of designating the plates of the genus *Peridinium* have been proposed. The designating method used in this paper is BROCH's (1910) which was partly modified by ROVIGNO and PAULSEN (1911) (Fig. 14). For the necessity of denoting the antapical intercalary plates of another genus, I used in thus paper φ, χ and w, and the first figure denoting each series of plates was put on the right shoulder of numerals, as, 7^{α} or 3^{γ} to indicate the number of plates comprised in the series.

---

* A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

Grateful acknowledgement is here made to Professor S. GOTO, of Tokyo Imperial University, for his kind advice to commence and continue this work, to Professor S. HOZAWA, of Tohoku Imperial University, for collecting material and supporting this work, and also to Professor S. HATAI, of Tohoku Imperial University, the Director of the Institute, for the permission to use the research table and for getting some reagents and apparatus from Sendai.

My sincere thanks to Professor M. KOIDZUMI, of Keio University, to Professor T. KABURAKI, of Tokyo Imperial University, and to Professor K. OKAMURA and H. MARUKAWA, of the Marine Fisheries Bureau for advice and kind help in getting literature, and also to Assintant Professor S. KOKUBO, to Assistant S. TAKATSUKI, and to Assistant Y. KAMADA, of the Asamushi Marine Biological Station, for collecting material.

Asterisks (*) were used through out the entire pages to refer to those works which I could not read the original papers.

DINOPHYSIS EHRENBERG.

1) **Dinophysis intermedia** PAVILLARD (?).

*PAVILLARD 1916, p. 58, Pl. III, Fig. 4.
FORTI 1922, p. 110, Pl. VII, Fig. 119.

An elongated egg-shaped species with deep cups composed of singular lists, and a rounded posterior end. The ratio of the length to the breadth is 0.32, and the widest part is about 2/3 from the anterior end.

Dimensions: Length, 57μ; transdiameter, 31μ.

Loc. Yunoshima, Aug. 16, 1926.

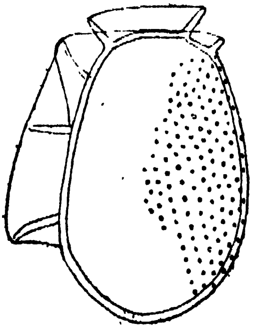


Fig. 1. *Dinophysis intermidia* PAVILL. (?) (×600)

2) **Dinophysis rotundata** CLAPARÈDE et LACHMANN.

* *D. rotundata* CLAP. et LACHM., 1859, p. 409, Pl. XX, Fig. 16.
SCHÜTT 1895, Pl. 1, Fig. 5.
PAULSEN 1908, p. 17, Fig. 18.

Body is broad oval in side view, with bilateral flattening. The ratio of the length to the breadth is 0.9. The plasma is light brownish yellow in colour.

Dimensions: Length, 53μ; transdiameter, 48μ.

Loc. Off Hadakaiwa, Aug. 14, 1926.

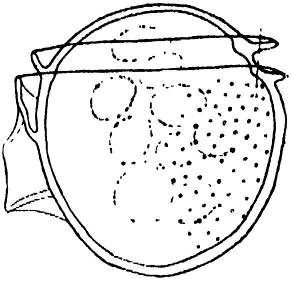


Fig. 2. *Dinophysis rotundata* CLAP. et LACH. (×600).

PHALACROMA STEIN.

3) **Phalacroma mitra** SCHÜTT.

SCHÜTT 1895, Pl. IV, Fig. 18.
SCHÜTT 1896, p. 26, Fig. 38.
OKAMURA 1907, p. 134, Pl. V. Fig. 43.
FORTI 1922, p. 105, Pl. VII, Fig. 109.

Dimensions: Length, 64μ; transdiameter, 55μ; dorsoventral diameter, 44μ.

Loc. Yunoshima, Aug. 8, 1926.

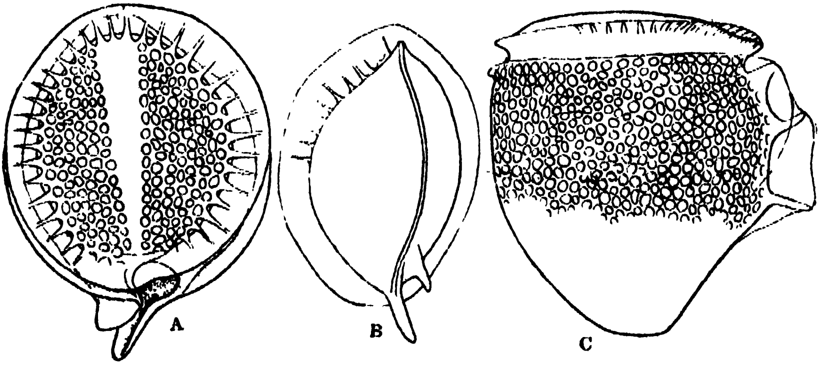


Fig. 3. a. *Phalacroma mitra* SCHÜTT.
A. Apical polar view. B, Antapical polar view. C, Side view.

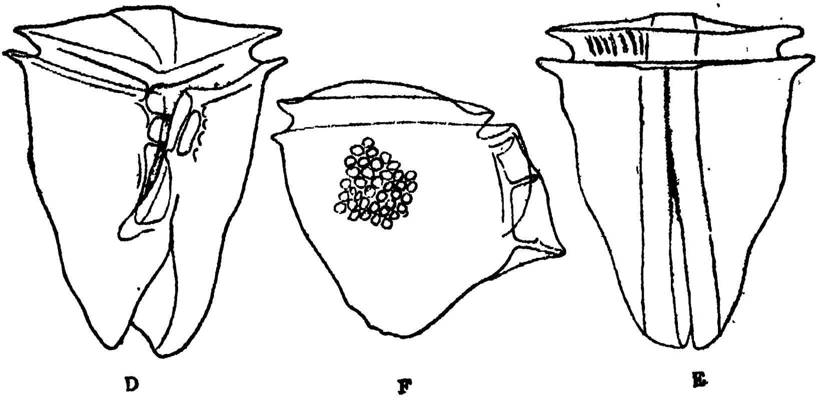


Fig. 3. b. D, ventral view. E, Dorsal view. F, Side view of another smaller specimen with a somewhat deformed contour. (×600)

DIPLOPSALIS BERGH.

4) **Diplopsalis lenticula** BERGH.

BERGH 1881, p. 244, Figs. 60–62.
SCHÜTT 1895, Pl. XV, Fig. 50.
SCHÜTT 1896, p. 21, Fig. 31.
OKAMURA 1907, p. 131, Pl. V, Fig. 44.
PAULSEN 1908, p. 35, Fig. 44.

The ventral area is short, flaring slightly posteriorly, guarded with a well developed list on its left and posterior rims.

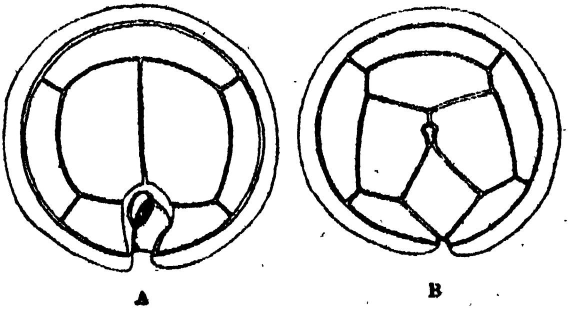


Fig. 4. *Diplopsalis lenticula* BERGH.

A and B, Diagrams of plates on the hypotheca and the epitheca. (×600)

Dimension : Transdiameter, 43μ.
Loc. Off Hadakaiwa, Aug. 10, 1926.

5) **Diplopsalis lenticula** BERGH var. (?).

This differs from the preceding species in having only one antapical plate and in it's smaller size. The ventral area is somewhat larger than the type species and is elongated obliquely and posteriorly to the left, along which left rim exhibits a broader list, but not extending to the posterior rim.

Dimensions : Length, 51μ ; transdiameter, 45μ.
Loc. Off Asamushi, Aug. 10, 1926.

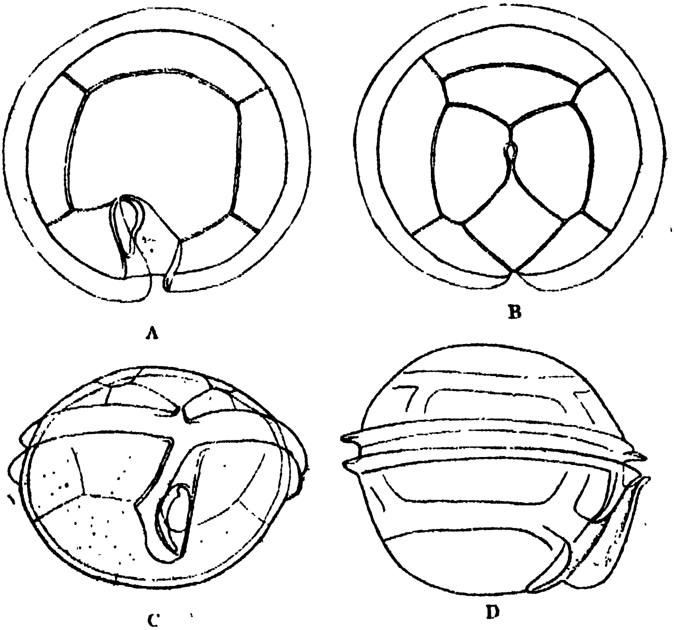


Fig. 5. *Diplopsalis lenticula* BERGH var?

A and B, Pattern of the plates the hypotheca and the epitheca. C, Ventral view. D, Side view. (×600)

GONYAULAX DIESING.

6) Gonyaulax polygramma STEIN.

*STEIN 1883. Pl. IV, Fig. 15.
SCHÜTT 1895, Pl. IIX, Fig. 33.
OKAMURA 1907, p. 132, Pl. III, Fig. 13.
PAULSEN 1908, p. 29, Fig. 36.
KOFOID 1911(a), p. 229, Pl. X, Figs. 6–7, Pl. XVII, Fig. 47.

A medium-sized, somewhat elongated species with longitudinally striated and reticulated surface. Body is rugged oval with both shoulders angled at the apical-precingular and postcingular-antapical sutures,

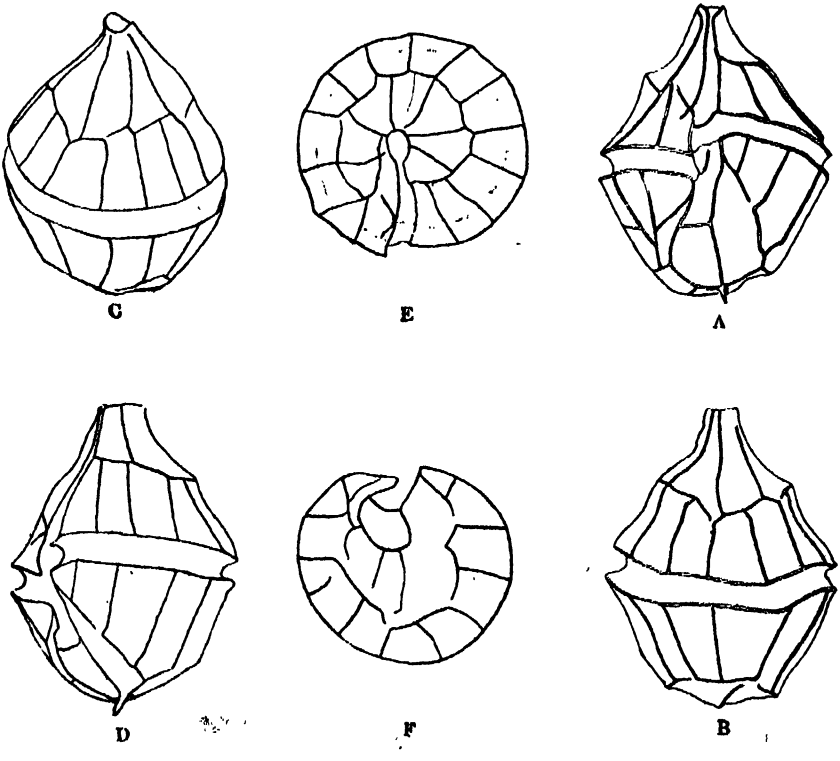


Fig. 6. *Gonyaulax polygramma* STEIN.

A, Ventral view. B, Dorsal view. C, Dorsal right-side view. D, Ventral left-side view. E and F, Apical and antapical view of a specimen showing the arrangement of the longitudinal ribs. (×600) bearing one or two small antapical spines. The longitudinal ribs vary in number and arrangement, but some of them are more or less exactly on the suture lines, so that it is possible to distinguish the thecal order from the arrangement of the ribs. The plasm is dark yellowish green.

Dimensions: Length, 57μ; transdiameter, 45μ; dorsoventral diameter, 42μ.

Loc. Off Hadakaiwa, Aug. 10, 1926.

7) **Gonyaulax spinifera** (CLAP. et LACHM).

PAULSEN 1908, p. 29, Fig. 37.
KOFOID 1911 (a), p. 209, Pl. X, Figs. 8-10, Pl. XVI, Fig. 39, Text-fig. A-D. (?)
G. mangini FAURÉ-FREMIET 1908, p. 230, Fig. 16.; Pl. XVI, Fig. 19.
* *Peridinium spiniferum* CLAP. et LACHM., 1895, p. 405, Pl. XX. Figs. 4, 5.

A minute species with a broad spiral girdle and an antapical horn, closely corresponding to *G. mangini* F. F. judging from the figure and descriptions given by the author.

Body is somewhat short and egg-shaped. The epitheca is subconical with a short apical horn. The girdle is very broad and median, with well developed lists of the body wall. The furrow is deeply impressed, and descending, and is displaced distally 3 girdle widths, and overlaps one of its widths. The groove connecting its two ends is narrow but is deeply impressed. The ventral area is a narrow furrow, curved somewhat obliquely to the right.

The hypotheca is a flat hemisphere, with a short but distinct antapical spine on its apex at the right post-margin of the longitudinal groove.

It is quite probable that *G. monoacantha* PAVILL is closely related to this species in its general features except in its large, distinct, apical horn.

Dimensions: Length, 27μ; transdiameter, 22μ.

Loc. Off Asamushi, Aug. 21, 1926.

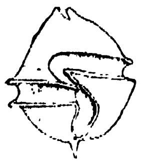


Fig. 7. *Gonyaulax spinifera* DIESING.
Ventral view. (× 600)

8) **Gonyaulax polyedra** STEIN.

*STEIN 1883, p. 13, Pl. IV, Figs. 7-9.
PAULSEN 1908, p. 31, Fig. 40.

Kofoid 1910, p. 238, Pl. XII, Figs. 16–20; Pl. XIV, Figs. 28, 29, 31; Pl. XVII, Fig. 48.
Conrad 1926, p. 95, Pl. II, Fig. 37.

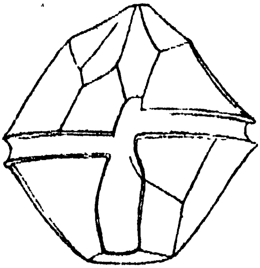


Fig. 8. *Gonyaulax polyedra* Stein. (×600)

A medium-sized species. The body is pentagonal with a truncated posterior end.

Dimensions: Length, 34μ; transdiameter 36μ.

Loc. Off Futagojima, Aug. 23, 1926.

9) **Gonyaulax turbynei** Murray and Whitting.

Murr. and Whitt. 1899, p. 323-324, Pl. XXIIX, Fig. 4.
Kofoid 1911(a), p. 225, Pl. XVII, Fig. 44.
Forti 1922, p. 80, Pl. VI. Fig. 67.

A small, ovoid species without a distinct apical horn. The surface is striated with pores which are sparsely but more or less longitudinally scattered, and the inter-striae regions are markedly reticulated.

Dimensions: Length, 40μ; transdiameter, 34μ.

Loc. Yunoshima, Aug. 16, 1926.

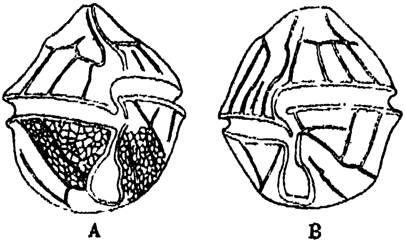


Fig. 9. *Gonyaulax turbynei* Mur. and Whitt.

A and B, Ventral views of two specimens. (×600)

PYROPHACUS Stein.

10) **Pyrophacus horologicum** Stein.

*Stein 1883, Pl. XXIV, Figs. 1–13. Pl. XXV, Fig. 1.
Bütschli 1885, Pl. LIV, Fig. 3.
Schütt 1895, Pl. XVII, Fig. 51.
Schütt 1896, p. 13, 15, Figs. 17, 21.
Paulsen 1908, p. 67, Fig. 89.

A large, flattened species. The number of plates are roughly proportional to its size, larger ones having a larger number of plates, and smaller ones a smaller number of plates. The specimen shown in Fig. 9, A and B is one having probably a minimum number of plates.

Dimensions: Length, 103-134μ; transdiameter, 94-124μ.

Loc. Off Asamushi, Aug. 16, 1926.

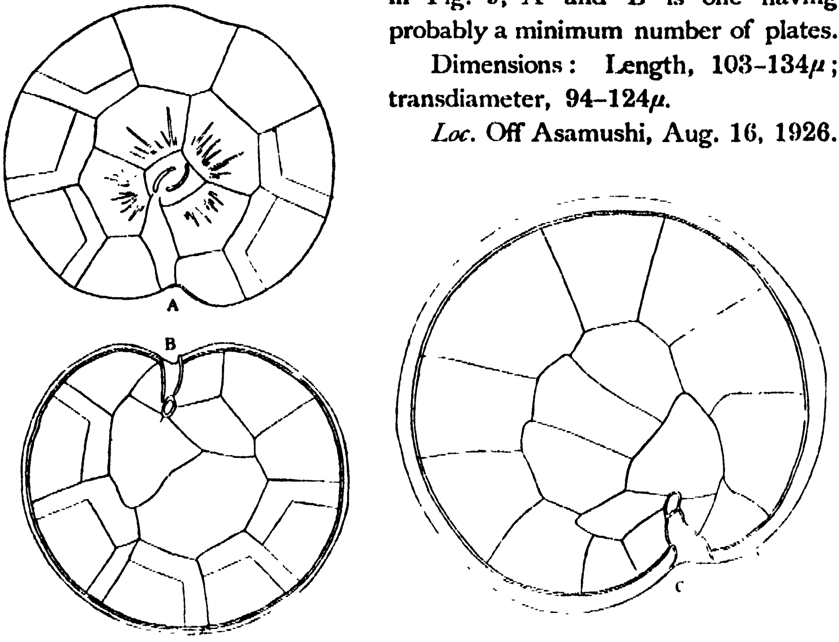


Fig 10. *Pyrophacus horologicum* STEIN.

A and B, Arrangement of the plates on the epitheca and the hypotheca of one specimen, having probably a minimum number of plates. C, The hypotheca of another larger specimen showing a larger number of plates. (×280)

SPHAERIDINIUM WOLOSZYNSKA.

11) **Sphaeridinium asymmetria**, n. sp.

A minute, spheroidal species with a somewhat curved ventral area, a relatively wide girdle, and no apical nor antapical processes.

The body is spherical. The epitheca and the hypotheca are both hemispherical. The most conspicuous character of this species is a distinct diagonal suture ridge on the epitheca from the left ventral to the right dorsal, crossing the apical pore. It is incomplete as it is only within the area of the apical and the intercalary plates, not extending into the precingular series which is only poorly developed.

It, then, divides the plates, though incompletely, into two groups, the apical *1*, *2*, and *4*, and the intercalary *γ* in the ventral, and the apical *3* and the intercalary *δ*, *ε*, in the dorsal half. Having some relation with the obliquity of the ridge, the plates of the right ventral and the left dorsal regions are larger or more extended than those of the left ventral and the right dorsal parts so that the apical plate *2* is smaller than the plate *4*, and the precingulars *a* and *b* are much smaller than the corresponding plates *g* and *f*. Of these the first surely is the smallest in the epitheca. The intercalary plate *δ* is the largest one, and the others, *γ* and *ε*, show a slight difference in size, but they are markedly displaced to the left side of the body by the asymmetrical development of the apical and the precingular plates. The precingular *c* is a very low but wide quadrangular plate. The apical pore is relatively large with a little smaller pore-like ventral slit. The slit is not accurately in the ventral median side, but is deflected about 45°. to the right side of the body from the dorso-ventral median plane. It also makes an angle of about 60°. with the diagonal suture ridge. The girdle is broad and median. The furrow is not deeply impressed, and is very slightly ascending without marked side-lists. The ventral area curves to the left and then to the right in sausage shape, bearing a semi-lunar list on its posterior right side. It is deeply impressed in the anterior part and along the right edge of the post-girdle part of the area, of which the middle portion is covered with the hyaline semi-lunar list. The hypotheca is hemispherical, as stated above. Its plate formula is 5^{A}, 2^{l}, and $2^{?}$. As will be clearly recognized in the text figures, one small, elongated plate, *χ*, is seen at the right of the incurved right rim, and a minute semi-lunar plate, *φ*, at the rear of the posterior edge of the ventral area. These small, hypothecal, intercalary plates, with the epithecal diagonal ridge, keep this species aloof from any other species of *Peridinium*. The surface is smooth and the plasm is colourless. The suture lines, excluding the ridge, are very narrow and indistinct, being recognized in some specimens only with the oil immersion.

This species is distinguished at once from *Peridinium globulus* and *P. minutum*, by its rounded apex. The curved ventral area and the irregularly displaced thecal plates serve to separate it from *P. sphericum* MURR. & WHITT. The ventral area of this species has some similarities

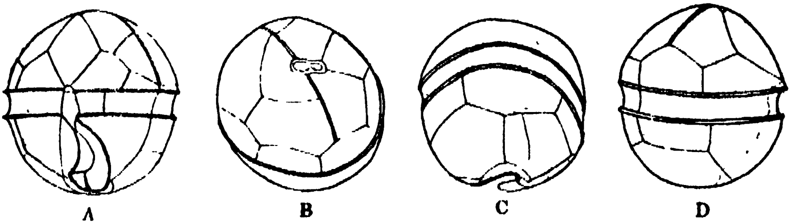


Fig. 11. *Sphaeridinium asymmetria*, n. sp.

A, Somewhat oblique ventral view. B, Oblique apical view. C, Oblique dorsal view of the hypotheca. D, Right side view. (×600)

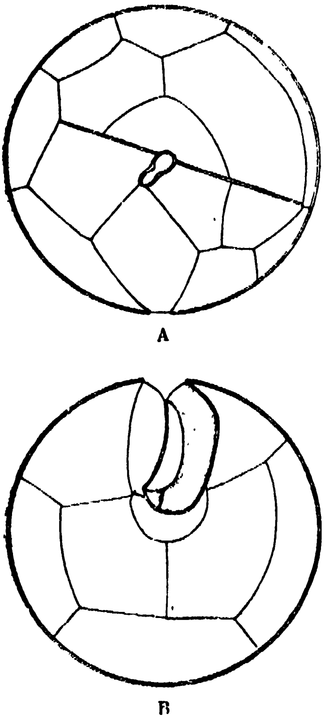


Fig. 12. *S. asymmetria*, n. sp.

A and B, Diagrams of the plate arrangement of the epitheca and the hypotheca. (×1500)

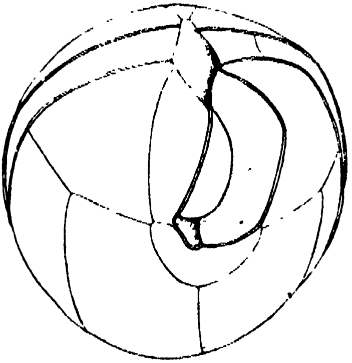


Fig. 13. *S. asymmetria*, n. sp.

Oblique antapical view of the ventral surface of the hypotheca. (×1500)

with *Goniodoma sphericum* MURR. & WHITT., which differs in having a smaller ventral area, a listed girdle with its proximal arch, and a different plate pattern. This species is remarkable both in having two antapical intercalaries and in the development of the apical diagonal band, as well as in the structure of the ventral area. It is not closely related structurally to any other species in the genus *Peridinium*, and its separation to the genus *Sphaeridinium* is justified mainly on the ground of its spherical body without girdle lists or any appendages except the ventral fin, and also on its antapical intercalary plates. The anterior antapical intercalary plate ought not to be regarded as a post-cingular plate, but must be separated into another series, the antapical intercalary series, with the minute posterior plate, from the view point of its development, so that the plates of the hypotheca consist of 5 precingular, 2 antapical, and 2 intercalary plates. The absence of one apical intercalary plate and the excess of one plate in the antapical intercalaries make it doubtful as to whether this species really deserves to be separated in the genus *Sphaeridinium* or not.

Dimensions: Length 36μ; transdiameter, 34μ.

Loc. Off Asamushi, Aug. 15, 1926. Very rare.

PERIDINIUM EHRENBERG.

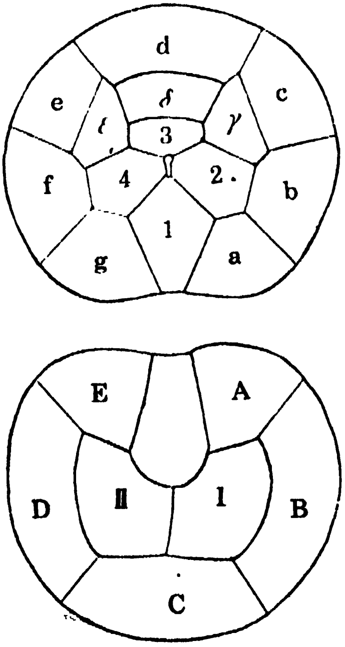


Fig. 14.

BROCH's modified method of denoting the thecal plates.

Upon attempting to arrange the vast divergencies of the morphological variations in the genus, early observers, SCHÜTT, BERGH, and GRAN divided the genus into two subspecies, *Protoperidinium* and *Euperidinium*, according to the anterior displacement of the girdle, coupled with the solid antapical horns for the former, and the posterior displacement of the girdle together with the hollow antapical horns for the latter. But in later year another system was proposed by JØRGENSEN (1912) on the basis of plate relationships, which was confirmed by BARROWS (1918), though criticized and somewhat modified by PAVILLARD (1916). BARROWS divided the genus into three groups, giving the name *Orthoperidinium* to those species having the ventral plate pattern of *P. achromaticum*, *Metaperidinium* to these having that of *P. pellucidum*, and *Paraperidinium* to those having the plate pattern of *P. spheroidea*. In the former system the main emphasis is placed upon the structures of the girdle and the antapical horn, but in the latter it is based only on the plate pattern without considering any of the characters of the appendages and the girdle. It would be convenient if the two systems were used together to denote the characters of the species, and the two are not unimportant in dividing the genus, each designating the different structural characters.

12) **Peridinium orbiculare** (PAULSEN) var. **temaris**, n. var.

A small, spherical species without elevation of the apical horn, or antapical spines.

The epitheca is subhemispherical. The plates are of the *Ortho-peridinium* type. The plates of the apical are well developed. The girdle is median, is relatively wide and ascending, and is displaced distally half its width. The furrow is not impressed, bearing low side-lists. The section at the girdle is circular. The ventral area is narrow and short, guarded by a broad, hyalinous, crescent fin on its left side, not extending to the posterior extremity. The hypotheca is also sub-hemispherical without post-indentation, or antapical spines. The antapical plates are large. A small, somewhat triangular, intercalary plate is on the right side of the ventral area as well as five postcingulars. The surface is smooth. The intercalary bands are wide and are provided with distinct transverse striations.

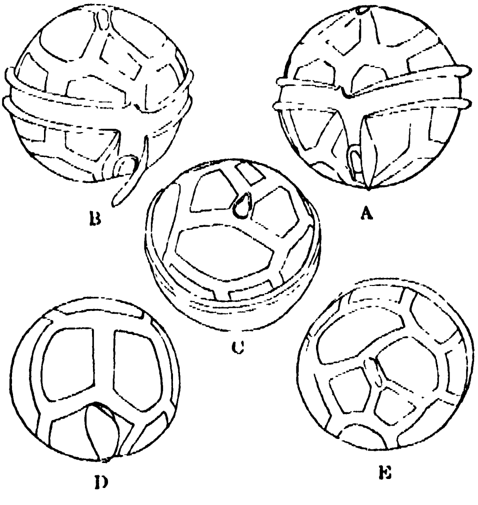


Fig. 15. *Peridinium orbiculare* (PAULSEN) var. *temaris*, n. var.

A, ventral view. B, Oblique ventral view. C, Dorso-apical view. D, Antapical view. E, Antapical view of the epitheca. (×600)

The lacking of one dorsal intercalary plate in this variety serves to separate this from the atlantic species.

Dimensions: Length, 35μ; transdiameter, 34μ.

Loc. Off Futagojima, Aug. 23, 1926.

13) **Peridinium nipponica,** n. sp.

A small, spherical species with a short apical horn and four delicate antapical spines.

The body is spherical without any ventral flattening. The girdle section is circular and is perpendicular to the body axis. The apical pore is at the terminal of the small apical horn with a short ventral slit. The thecal plates are of the *Paraperidinium* type with three intercalary plates. The epitheca is hemispherical, spmmetrical with the hypotheca. The apical plate $\imath$ is a large hexagonal. The precingulars b and f are the largest in the series, while the dorsal d is a flat plate. The dorsal, hexagonal, intercalary δ is as large as the plate h, but the other two pentagonals, γ and ϵ, are the smallest in the epitheca. The intercalary bands are wide. The girdle is median and is relatively narrow, with hyaline side-lists. The furrow is circular and is faintly impressed. The ventral area is spoon-shaped, comprising anterior narrow and posterior wider portions. The flagellar pore is in the left corner of the posterior wider portion, the ventral and the dorsal rims of which bear two short spines. Two other longer spines are seen on the postero-lateral edges of the area, all of them having no side-fins except the short ventral one. A longitudinal fin is on the left side of the ventral area connecting the short, dorsal spine and the proximal part of the posterior girdle list. The longer spine is 0.33 times that of transdiameter. The surface is smooth except the intercalary bands, which are faintly striated transversely. The plasm is colourless. A closely resembling species, *P. sphericum*, was reported off the coast of Tosa, Shikoku, by Okamura, but differs from this species in its larger size, slightly ascending girdle, and two finned antapical spines.

It is closely related to *P. ovum* in general, but differs in thecal plates and some structures in the posterior region of the ventral area.

Dimensions: Length, 40μ; transdiameter, 36μ.

Loc. Off Asamushi, Aug. 16, 1926.

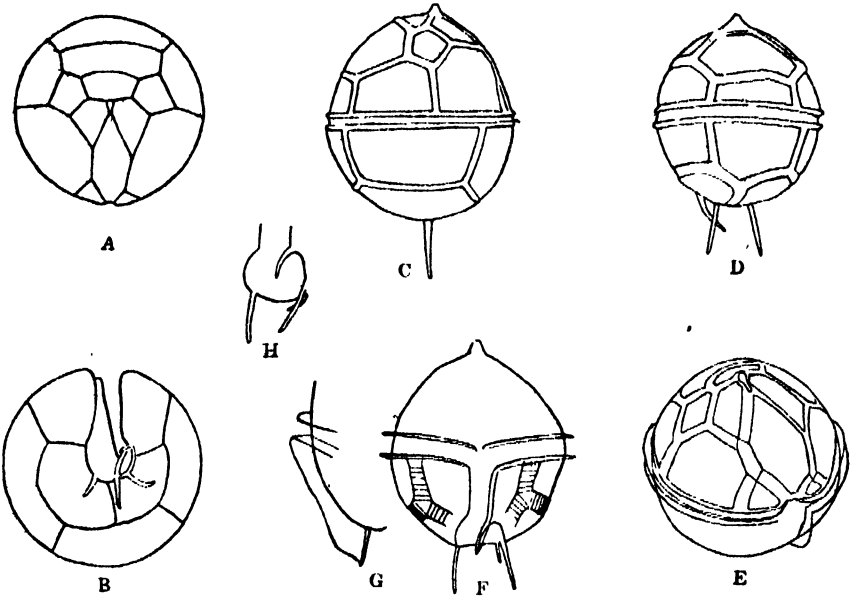


Fig. 16. *Peridinium nipponica*, n. sp.

A and B, Diagrams of the plates on the epitheca and the hypotheca. C, Side view. D, Dorsal view of another specimen. E, Oblique polar view of the specimen C. F, Ventral view of another one. G, Side view of the longitudinal fin of the ventral area. H, Oblique antapical polar view of the posterior ventral area, showing the four spines. (×600)

14) **Peridinium spheroidea**, n. sp.

A small, spherical species with two finned antapical spines and broad girdle lists. The body is spherical without ventral flattening, so that both the epitheca and the hypotheca are hemispherical. The apical horn is small and flat, hard to detect. The apical pore is small with a short ventral furrow. There seems to be a ventral slit, but close observation proves it to be not a slit but a groove. The intercalary bands are broad and transversely striated. The plates are of the *Metaperidinium* type with three intercalaries. The apical *1* is a large hexagonal plate, but the dorsal plate *3* is small. The lateral intercalary plates γ and ϵ are small, and the dorsal δ is somewhat larger. The ventral precingular *a* and *g* are minute, triangular plates. The girdle is ascending and is displaced distally 1.5 its width, with a proximal arch. The furrow is relatively narrow, faintly depressed, with broad side lists bearing abundantly ribbed fins on their inner sides. Short, numerous, perpendicular wrinkles, their length being shorter than one-third of the girdle breadth, are observed along both sides of the groove, which are only the traces of the rib's bases in the lists. The girdle plane is not perpendicular to the body axis, but is inclined about 15°. from the perpendicular plane. The ventral area is a narrow groove guarded with side fins. The right side fin is a little narrower than the left, while the left one is broad and curves inwardly, spreading over and nearly covering the left half of the area. The flagellar pore is in the left side, near the truncated posterior margin, of the area. Two long, antapical spines, each with three lateral fins are on the antapex of the hypotheca, one on the right corner of the truncated post-margin of the ventral area, and the other on its left edge a little distance from its left corner. The left spine inclines ventrally. The left antapical plate 1 is smaller than the right one. The surface is smooth.

Three spine-like processes are observed at the antapex in ventral view, but the shorter left one is not really a spine, but merely an image caused by the lengthwise view of the posteriorly thickened part of the side fin at the intersection of this with the left median fin of the spine, which is not noticed in side view.

This species is distinguished at once from *P. globulus*, by its different thecal arrangement and in having antapical spines. *Peridinium cerasus*, and *P. orbiculare* are distinguished from this by their smaller size, and its antapical spines serves to separate this from *P. orbiculare*. *Peridinium sphericum* OKAMURA is one of the best related species, but differs in its much smaller size, in having the equatorial girdle plate perpendicular to the body axis and in having a distinct apical horn. On the other hand, the distal displacement of the girdle, the shorter but spreading left antapical spine and the broad side list on the left edge of the ventral area show the close relation of the two. OKAMURA's non-detailed figures and descriptions make it difficult to identify it with this species, and it is quite probable that they are two different species.

Dimensions: Length, 62μ; transdiameter, 58μ; dorso-ventral diameter, 54μ.

Loc. Off Futagojima, Aug. 28, 1926.

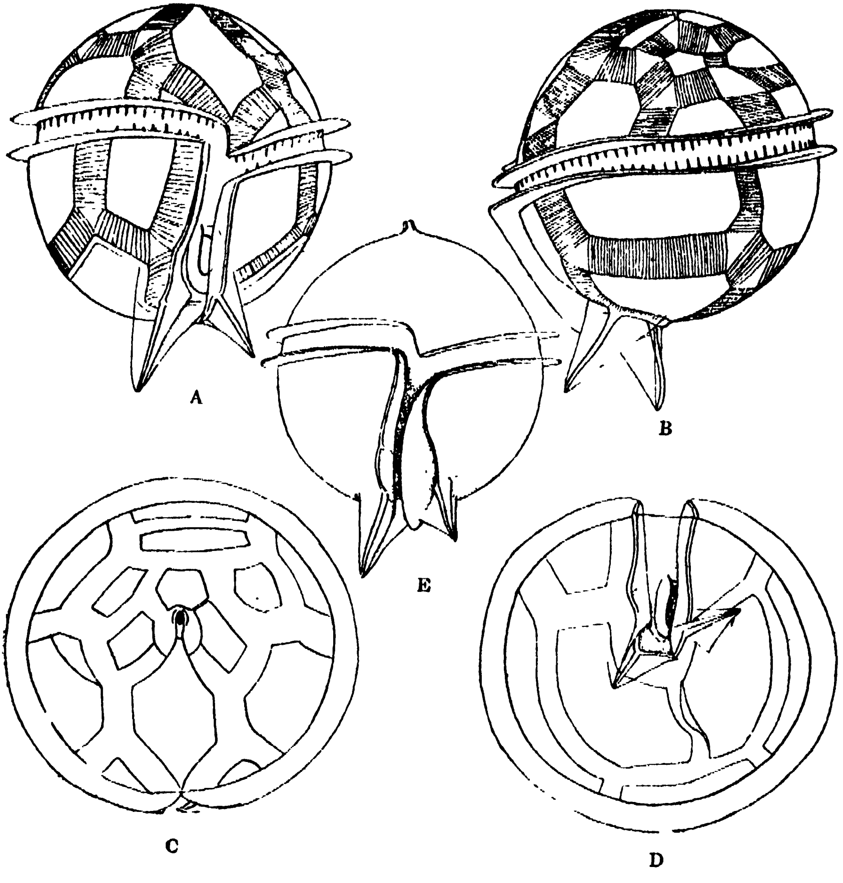


Fig. 17. *Peridinium spheroidea*, n. sp.

A, Antapical ventral view. B, Side view. C and D, Pattern of the plates of the epitheca and the hypotheca. E, Ventral view of another smaller specimen. (×600)

15) **Peridinium hirobis**, n. sp.

A minute species with an extraordinarily wide girdle and two antapical spines. The epitheca is subhemispherical, the anterior surface of which is contracted to a short apical horn. The plates are of the *Metaperidinium* type with three dorsal intercalaries. Its cross-section at the girdle is broad oval. The girdle is post-median, and is very wide, with ribbed lists. Its proximal part is far wider than the distal part. The furrow is not deeply impressed, and is slightly ascending distally. The ventral area is also wide, widening posteriorly, guarded with a broad fin on its left side. The flagellar pore of this species is very large, is elongated along its left side and extends anteriorly to the post-margin of the proximal girdle end. The hypotheca is a flat subhemisphere with a shallow post-indentation at the post-margin of the ventral area. In addition to the two long antapical spines, slightly diverging posteriorly, a short spine is seen on the inner side of the left spine in the ventral view. It is not a spine, but an image formed by the posterior extension of the side list. The surface is smooth and the intercalary bands are narrow and indistinct.

Dimensions: Length, 26μ; transdiameter, 24μ; dorsoventral diameter, 22μ.

Loc. Off Futagojima, Aug. 23, 1926.

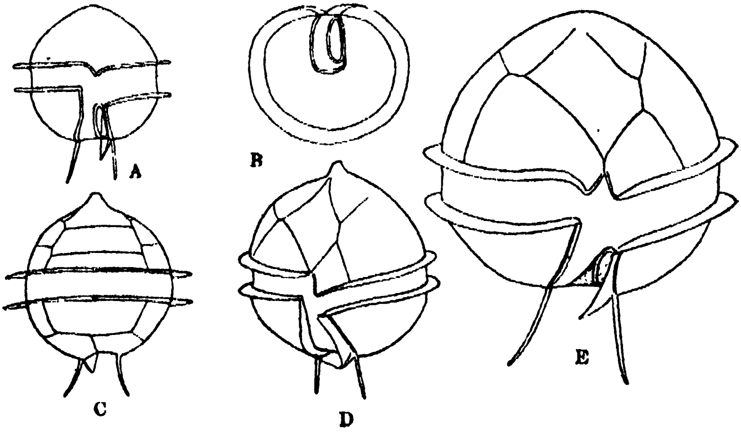


Fig. 18. *Peridinium hirobis*, n. sp.

A, Ventral view of a smaller specimen with a broken apical part. B, Antapical view. C, Dorsal view of another larger one. D, Oblique ventral view. (×600) E, Ventral view of a different, imperfect specimen. (×1500)

16) Peridinium pellucidum (BERGH).

SCHÜTT 1895, Pl. XIV, Fig. 45.
AURIVILLIUS 1898, p. 98.

PAULSEN 1908, p. 49, Fig. 61.
BROCH 1910, p. 188, Fig. 6.
Protoperidinium pellucidum BERGH 1881, p. 227, Figs. 46-48.

A small, globular species with a tapering apical horn, two somewhat thick antapical spines and a broad girdle.

The epitheca exceeds the hpyotheca. The epitheca is subhemispherical, the side of which contracts gradually into a short apical horn, bearing a long ventral slit along its ventral anterior end. The plates are of the *Metaperidinium* type with three intercalaries. It is nearly circular at the girdle. The girdle is broad, not impressed, bearing wide, coarsely ribbed lists. It is ascending, and is displaced distally half its width. The ventral area widens somewhat posteriorly, invading deeply, posteriorly, the antapical plates. The furrow is deeply excavated, its posterior margin much indenting the antapex. It is guarded with a broad lunar fin on its left side. The hypotheca is subhemispherical with two antapical spines on its antapex. The spines are subequal, often provided with fins along their whole length or at basal parts alone, their basal parts, thickening proximately, abruptly merge into the body contour. The surface is smooth. The intercalary bands are narrow but distinct. A minute pore-like figure is observed in the band anterior to the anterior plate of the ventral area. The thecal wall is thin.

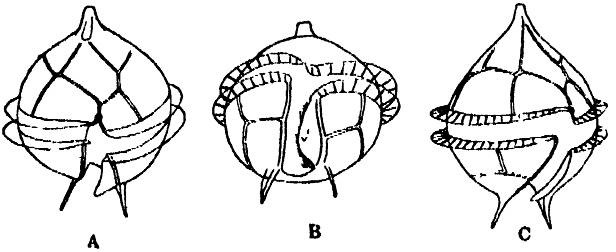


Fig. 19. *Peridinium pellucidum* (BERGH)

A, Ventral view. B, Ventral antapical view. C, Right ventral view. (×600)

The apical horn of this species is shorter and less distinctly differentiated than that of *P. steini*, but is more pronounced than *P. breve*. Of this species, the apical horn is more slender, the body is larger and somewhat elongated (the ratio of breadth to length is 1.3) than those of BROCH's Val di Bora specimens to which it is most closely related.

Dimensions: Length 34μ (including apical horn); transdiameter, 31μ.

Loc. Off Futagojima, Aug. 23, 1926.

17) **Peridinium okamurai**, n. sp.

P. pallidum PAULSEN var (?), OKAMURA 1912, p. 14, Pl. IV, Fig. 70.

A small, elongated, pear-shaped species with a short apical horn and two finned antapical spines.

The body is slightly dorso-ventrally compressed. The epitheca has convex sides which contract abruptly to a short apical horn. The apical pore bears a wide but short ventral slit. The plates are of the *Paraperidinium* type with three intercalary plates. The dorsal intercalary plate δ is larger than the apical *3*, and its surface in some specimens is markedly convex so as to form a hump in the middle of the dorsal side. Consequently, the apical plate *d* can not be observed in the apical view of the epitheca, being hidden under the hump in such a specimen. The girdle is post-median and ascending, and is displaced distally one girdle width. The furrow is not deeply impressed, bearing broad, well developed, ribbed side lists with a large rib at each of the bases of the longitudinal intercalary bands. The ventral area is narrow without posterior widening, guarded with the left broad and right low side lists. It noticeably indents the antapicals. The section at the girdle is broad oval without distinct ventral flattening. The hypotheca is rounded posteriorly with a narrow and slight post-indentation, each bearing two finned, long, antapical spines on its postero-lateral corner, which expand posteriorly. The antapicals are deeply indented by the ventral area, and also by the dorsal postcingular plate *C*.

The surface is smooth. The intercalary bands are broad and striated.

The girdle plane being perpendicular to the major axis, the long spreading antapical spines, the distinct apical horn distinguish it from *P. pallidum* OSTENFELD. This species is distinguished from *P. michaelis*, by its spreading spines, swollen epitheca, different type and number of thecal plates and by having no proximal arch of the girdle.

Dimensions: Length, 59–75μ; transdiameter, 38–50μ; dorso-ventral diameter, 34–40μ.

Loc. Off Futagojima, Aug. 23, 1926.

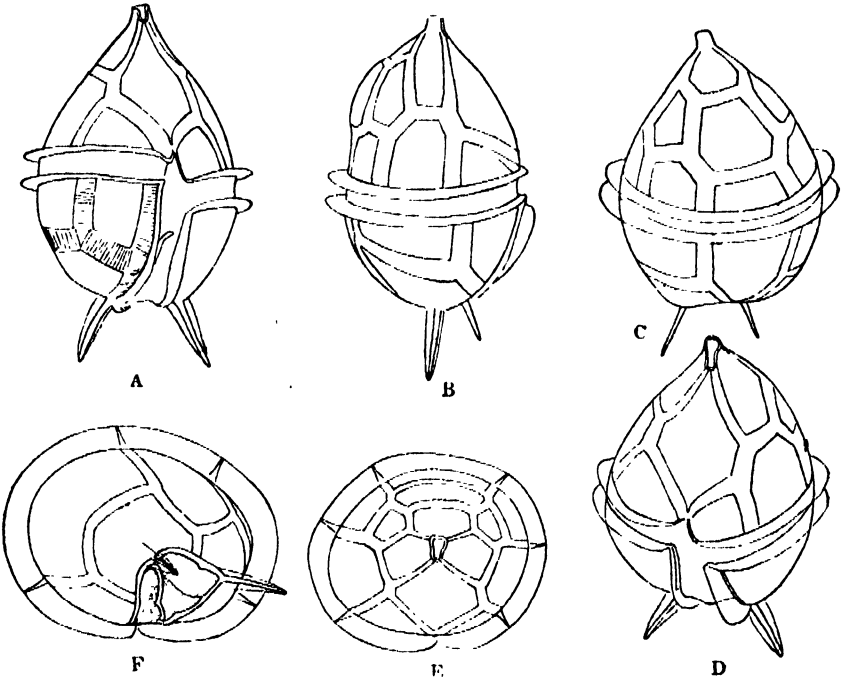


Fig. 20. *Peridinium okamurai, n.* sp.

A, Ventral view. B, Side view. C, Oblique dorsal view. D, Antero-ventral view. E and F, Apical and antapical (oblique) view. (×600)

18) **Peridinium oceanicum** VANH. v. **oblongum** AURIV.

AURIVILLIUS 1898, p. 96.
SCHÜTT 1895, Pl. VIII, Fig. 44 (?).
PAULSEN 1908 p. 55, Fig. 70.
BROCH 1910, p. 190, Fig. 7.
FORTI 1922, p. 90, Pl. VI. Fig. 83.

A spheroidal species with long or short apical and antapical horns and a flat list along the right ridge of the longitudinal furrow.

Dimensions: Length, 90–120μ; transdiameter, 60–65μ; dorsoventral transdiameter, 50μ.

Loc. Off Asamusni, Aug. 21, 1926.

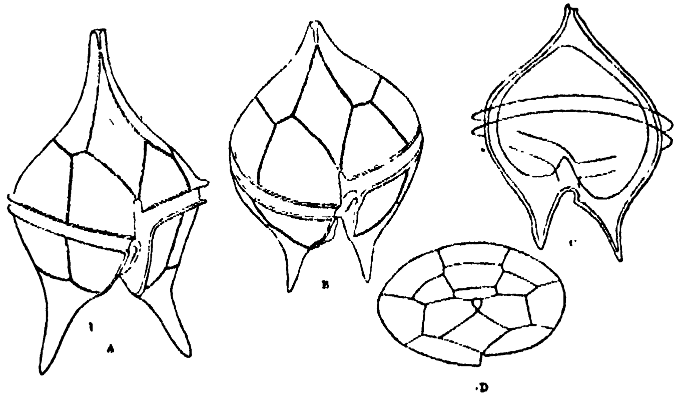


Fig. 21. *Peridinium oceanicum* VANH. v. *oblongum* AURIV.

A, Somewhat elongated specimen. B and C, Rotund specimens. D, Arrangement of the plates on the epitheca. (×280)

19) Peridinium adriaticum BROCH.

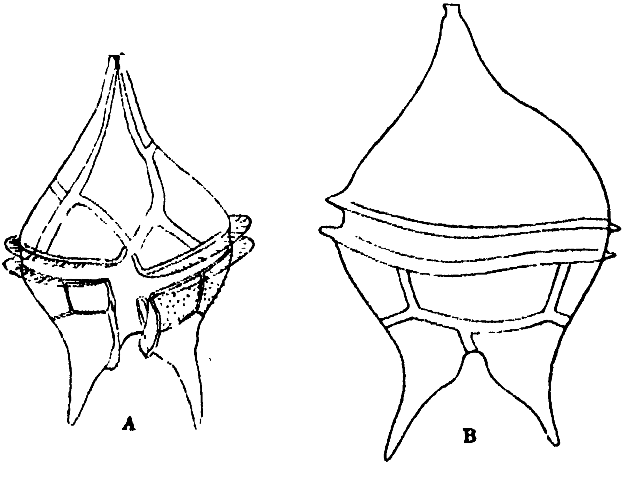


Fig. 22. *Peridinium adriaticum* BROCH.

A, Ventral view. B, Dorsal view of another specimen. (×600)

BROCH 1910, p. 191, Fig. 8.
FORTI 1922, p. 91, Pl. VII, Fig. 84.

This species is akin to *P. oceanicum* except that its ascending girdle is displaced distally one girdle's width. The plates are of the *Metaperidinium* type. The surface is reticulated with small thorns in their nodes. The longitudinal furrow is guarded laterally with a well developed left and a flat right fin.

Dimensions: Length, 75–81μ; transdiameter, 47–51μ.

Loc. Off Asamushi, Aug. 15, 1926.

20) **Peridinium koma**, n. sp.

A small, trochoidal species with a broad ventral area and an asymmetrical antapical horn.

The body is broad peg-top form with an eccentric antapical horn. The epitheca is conical, of which the anterior sides gradually merge into the apical horn. The plates in the epitheca are of the *Orthoperidinium* type with three intercalaries. The apical *1* is a slender plate and the other *2*, *3*, and *4* are relatively small. In the intercalaries dorsal *δ* is larger than the rest. The apical *a* and *g* are elongated plates, but the plates *b*, *f*, and *c*, *e* diminish their altitudes in order, the dorsal *d* is the shortest in its altitude. The girdle is narrow and post-median. The furrow is not deeply impressed, descending with the distal arch, and is displaced distally its own width. The ventral area is very broad and subterminal. The hypotheca is subhemispherical, bearing a prominent antapical horn a little to the right of the median axis. There is a slight elevation on the left side of the post-margin suggestive of a left rudimentary antapical horn.

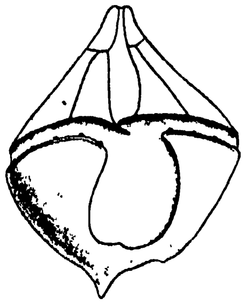


Fig. 23. *Peridinium koma*, n. sp. (×600)

No corresponding species has been reported as far as I know, except *Heterocapsa pacifica* which has some resemblances in having the asymmetrical horn and the slight rudimentary elevation, though on the opposite side.

Dimensions: Length, 57μ; transdiameter, 47μ.

Loc. Off Asamushi, Aug. 15, 1926.

21) **Peridinium conicum** (GRAN).

*OSTENFELD u. SCHMIDT 1900, p. 174.
OKAMURA 1907, p. 132, Pl. V, Fig. 36.
PAULSEN 1908, p. 58, Fig. 74.
BROCH 1910, p. 195, Fig. 11.
FORTI 1922, p. 88, Pl. VI. Fig. 79.
P. divergens pyramidale KARSTEN 1907, Pl. XXIII, Fig. 14.

A short and broad species with distinct, conical, antapical horns. The epitheca is broadly conical, with slightly concave or nearly straight sides. The cross section at the girdle is reniform. The antapical horns are formed by the deep post-indentation of the posterior part of the ventral area, and are somewhat laterally compressed. Judging from many observations, there is a tendency that the precingular-apical intercalary bands in the ventral and the precingular-intercalary intercalary bands in the dorsal area to be less developed than the others, so that only these bands between two adjacent plates in one and the same series and also the apical-intercalary intercalary bands are distinctly detected.

Dimensions: Length, 95μ; transdiameter, 90μ.

Loc. Asamushi, Aug. 15, 1926.

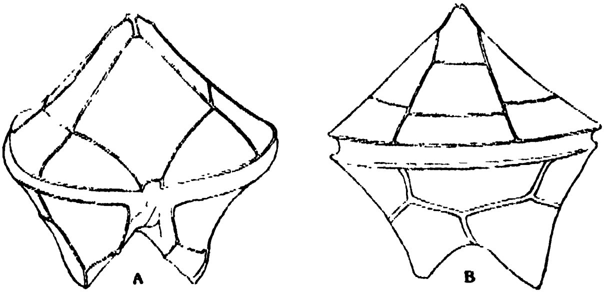


Fig. 24. *Peridinium conicum* (GRAN).
A, Ventral view. B, Dorsal view. (×280)

22) **Peridinium conicum** (GRAN) forma **asamushi**, n. form.

This differs from the preceding species in having two dorsal intercalary plates. The dorsal intercalary plate δ is divided by a longitudinal band into two unequal plates, the larger left and the smaller right.

Dimensions: Length, 105μ; transdiameter, 107μ.

Loc. Off Asamushi, Aug. 13, 1926.

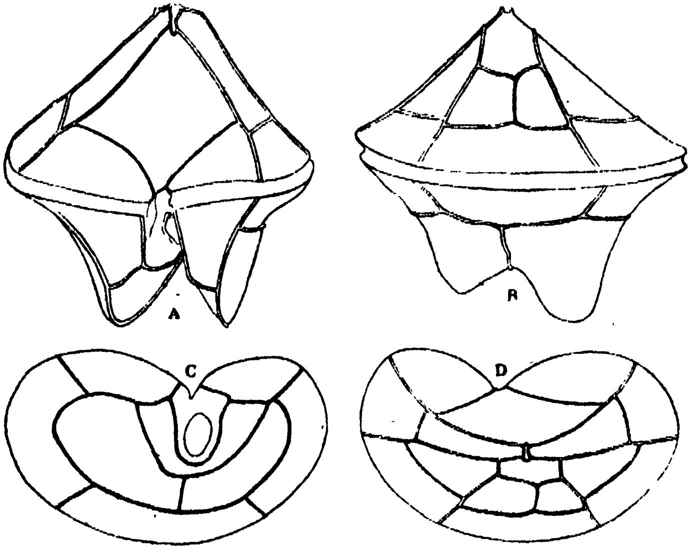


Fig. 25. *Peridinium conicum* (GRAN) forma *asamushi*, n. form.

A, Ventral view. B, Dorsal view. C and D, Arrangement of the plates on the hypotheca and the epitheca. (×280)

23) **Peridinium crassipes** KOFOID.

KOFOID 1907(e), p. 309, Pl. XXXI, Figs. 46, 47.
PAULSEN 1908, p. 58, Fig. 73.
BROCH 1910, p. 193, Figs. 9, 10.
MANGIN 1910, Pl. VIII, Figs. 1–5.
FORTI 1922, p. 92, Pl. VII, Fig. 86.

A medium-sized, broad species with blunt short antapical horns, and the proximal arch of the girdle. The cross section at the girdle is broad reniform, not so dorso-ventrally flattened. Two or three small protuberances are observed in the ventral view on the top of the semi-truncated end of the antapical horn, the outermost of them is the longest and corresponds to the real tip of the horn. The one or two inner processes are not the real spines, but of the posterior extensions of the lists of the longitudinal furrow and that along the rim of the longitudinal intercalary bands. The antapical horn is not dorso-ventrally thickened, but on the contrary flattened without forming dorsal and ventral angles, as the San Diego specimens. The slight concavity of the sides of the epitheca shows an intermediate form between form *typica* and form *autumnalis*.

Dimensions: Length, 87μ; transdiameter, 93μ.

Loc. Off Hadakaiwa, Aug. 8 and 29, 1926.

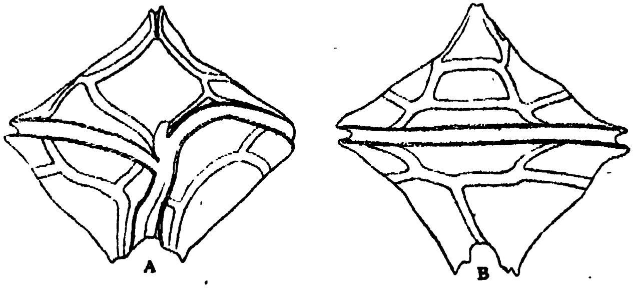


Fig. 26. *Peridinium crassipes* Kofoid.
A, Ventral view. B, Dorsal view. (× 280).

24) **Peridinium crassipes** Kofoid forma (?).

This form differs from the preceding in having a different plate arrangement, less pronounced proximal arch, and distal displacement

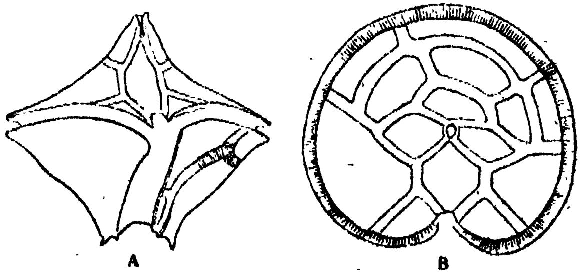


Fig. 27. *Peridinium crassipes* Kofoid forma (?).
A, Ventral view. B, Apical view. (× 280)

of the girdle and more concave epithecal wall. The plates of the epitheca are of the *Paraperidinium* type with distinctly displaced dorsal plates to the left.

Dimensions: Length, 78μ; transdiameter, 81μ.

Loc. Off Hadakaiwa, Aug. 8, 1926.

25) Peridinium pentagonum GRAN.

*GRAN 1902, p. 185, 190.

PAULSEN 1908, p. 59, Fig. 77.

CONRAD 1926, p. 96, Pl. II. Figs. 41, 42.

P. divergens pentagonum KARSTEN 1907, Pl. XXIII. Fig. 11.

Dimensions: Length, 64μ; transdiameter, 75μ.

Loc. Off Hadakaiwa.

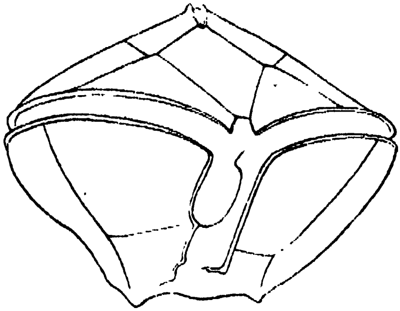


Fig. 28. *Peridinium pentagonum* GRAN. (×280)

26) Peridinium pentagonum GRAN forma depressum, n. form.

A broad and flattened form of *P. pentagonum*. The girdle is nearly circular, not showing the strong proximal arch and distal displacement. Body is strongly flattened dorsoventrally, so that the cross-section at the girdle is a flat reniform, which makes the body very transparent and hyalinous. The intercalary bands in this form are irregularly developed. The same relations as already pointed out in *P. conicum* are also noticed in this form in a more pronounced degree, so that in the apical view only three lateral suture lines with two short dorsoventrally connecting bands near the apex are seen with distinctness while others are not worked out even with close examination, except one or two suture lines which are only surmised through the somewhat lineally arranged ridges of the fine polygonal meshes on the reticulated surface. It seems an extreme case of the irregular development of the band seen in *P. conicum*. In the hypotheca only one lateral line is prominent, probably these between *A-B* and *D-E*. This type of irregularity is wholly different from that described by BROCH in *P. conicum*.

Dimensions: Length, 72μ; transdiameter, 87μ; dorso-ventral diameter, 35μ.

Loc. Asamushi, Aug. 20, 1926.

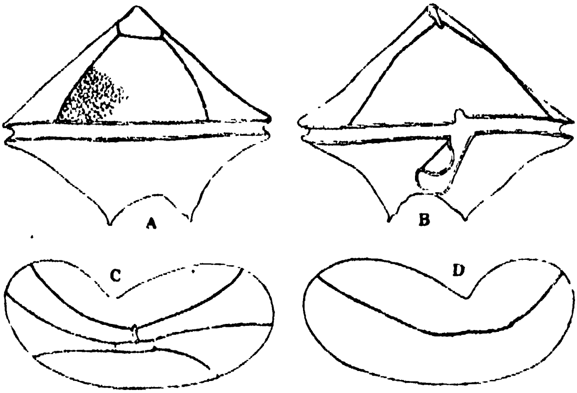


Fig. 29. *Peridinium pentogonum* GRAN forma *depressum*, n. form.

A, Dorsal view. B, Ventral view. C and D, Suture lines of the epitheca and the hypotheca. (×280)

27) **Peridinium quinquecorne,** n. sp.

A small, peculiar species with four antapical spines on the post-margin.

Body is rotund posteriorly and pointed anteriorly. Its length is 1.3 transdiameter.

The epitheca is subconical with an indistinct short apical horn. The apical pore has a short ventral slit with a small finger-like protuberance projecting on its dorsal wall. The plate formula of the epitheca is 4^l, 2γ, and 7^a. The ventral precingular plates *a* and *g* are the largest while the two dorso-lateral intercalaries are the smallest. The lateral apical *2* and *4* are somewhat concave, forming the side walls of the apical horn. The dorsal apical *3* is a somewhat large septagonal and is directly adjacent to the small precingular *d*. The girdle is comparatively wide and is deeply impressed with distinct side lists of the body wall. It is quite post-median, so that the epitheca exceeds the hypotheca, excluding the spines, the ratio of the altitudes of epitheca to hypotheca being 0.53. The furrow is slightly descending, and is displaced distally its half width without a marked proximal arch. The section at the girdle is flattened reniform. The hypotheca is somewhat polygonal in ventral view, four spines on each angle. The postcingular plates are low in altitude while the major part of the hypotheca is covered with two antapicals, the spines being on the latter.

The central right spine is displaced dorsally while the rests are middle or slightly ventral, but the central left is the longest of the four though not so distinct as in *Gonyaulax triacantha.* The spines, except the right-most one, are slightly curved inwardly or ventrally. The ventral area is broad and subterminal resembling much that of *G. triacantha.* The surface is smooth without any markings.

Gonyaulax triacantha is the sole species which has a close resemblance to this species in having many antapical spines and a flattened girdle section. The first glimpse of this specimen made me conjecture that it belongs to *G. triacantha,* but close examination of the general shape, the arrangement of the plates, the structure of the girdle, and the ratio of the epitheca to the hypotheca have proved it to belong to a wholly different genus *Peridinium.* No corresponding form was found in the genus.

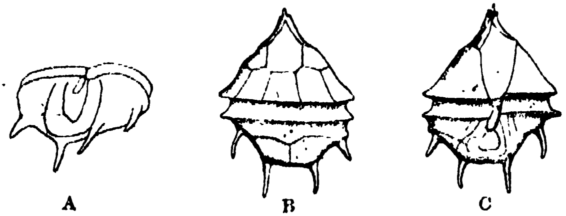


Fig. 30. *Peridinium quinquecorne,* n. sp.

A, Ventral polar view of the hypotheca of an incomplete specimen. B, Dorsal view of another specimen. C, Ventral view. (×600)

Fuller accounts will be given when I could get enough material to further analyse their structures as I have here observed only two individuals, one incomplete and one complete one.

Dimensions: Length, 30μ (excluding antapical spines); transdiameter, 26μ.

Loc. Off Hadakaiwa, Aug. 11, 1926.

28) Peridinium achromaticum LEVAND.

*LEVANDER 1902, 49, Figs. 1, 2.
OSTENFELD 1908, p. 165, Pl. V. Figs. 29-30, 40-43.
PAULSEN 1908, p. 62, Fig. 80.

A small species with a broad girdle, rounded hypotheca and bluntly pointed epitheca. The epitheca is subconical, bearing a long ventral slit of the apical pore at the apex. The section at the girdle is approximately circular. The hypotheca is rounded without ventral flattening, but the ventral area is deeply excavated which widening and deepening posteriorly extends to the post-margin.

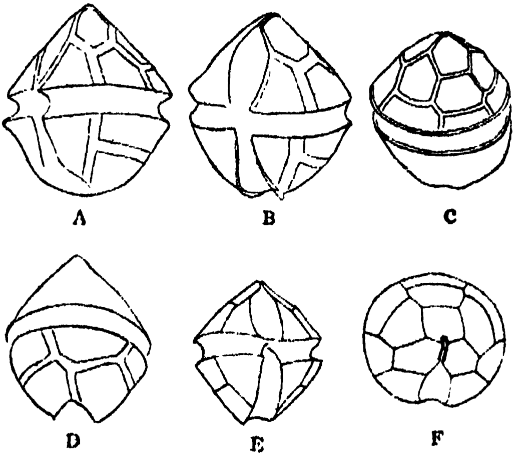


Fig. 31. *Peridinium achromaticum* LEV.

A and B, Left ventral view. C, Left dorsal view of another specimen. D, Dorsal antapical view showing the asymmetrical dorsal postcingular *C* and the displaced intra-antapical suture. E, Ventral view of a different specimen. F, Epithecal view of the pattern of the plates in the C specimen. (×600)

The furrow is guarded by a low fin on each side, which broadens posteriorly. A minute spine-like process is seen in the ventral view on each side of the furrow at the antapex which is not a spine but the image of the posterior extension of the side fin formed in its lengthwise view. The surface is smooth, except the broad intercalary bands in which faint transverse striations are observed.

Its epithecal plates are 14 in number having three intercalaries, which agrees with the LEVANDER's Finland specimens.

Dimensions: Length, 28-38μ; transdiameter, 24–31μ.

Loc. Off Futagojima, Aug. 23, 1926.

29) **Peridinium levanderi,** n. sp.

A small, broad rhombic species with a wide girdle. The body is a rounded square in ventral view with somewhat pointed apical and broad antapical ends. Its length is slightly longer than the width, being 1.1 transdiameter. The epitheca is broad conical with slightly convex sides. The apical pore is small with a long ventral slit. The apical *1* is an elongated plate, and the small hexagonal *2* and *4* make the apex triangular in cross-section with the quadrangular dorsal apical *3*. The intercalaries γ and ϵ are of unequal size in the middle in the dorsal surface in saddle-fashion, comprising the larger left and the smaller right to the reversal result of the size of the precingulars *c* and *e*. The ventral precingulars *a* and *g* are tall, trapezoidal plates, measuring about 0.76 altitudes of the epivalva. The plates *b* and *f* have nearly the same altitude but are much broader, and the dorsal *d* is a small, flat, pentagonal plate. The girdle is broad, slightly descending, and is displaced distally one-third of its width. The furrow is deeply impressed with low side lists. The hypotheca is somewhat dorso-ventrally flattened posteriorly, and the post-margin of the ventral area is indented slightly, leaving the lateral part of it in two blunt antapical horn-like processes. The ventral area is narrow without marked posterior widenings. The surface is smooth and the plasm is colourless. The intercalary bands are narrow.

I am in doubt whether this species is the one just corresponding to *P. achromaticum* L. LEVANDER reported 14 plates in the epitheca in the Finland specimens, while OSTENFELD found and figured only 13 plates in the Aral specimens of *P. achromaticum*, which has two separated intercalary plates. Our specimens correspond to the latter in having 13 plates in the epitheca, but differ from it in the relative size of the plates and the arrangement of the intercalaries. In the Aral and the Finland specimens, the apical plates *2*, *3*, and *4* are comparatively larger and the intercalaries smaller as compared with our Mutsu specimens, so that the anterior ends of the precingular plates *a* and *g* of the former are far behind the corresponding lines of our specimens in ventral view. The ventral area is narrow in the Aral and broad in the Finland specimens (according to PAULSEN's figure), while in ours both types are detected.

No antapical spines, reported both from the Aral and the Finland specimens, were observed in our specimens. Notwithstanding these differences, there is no doubt of the existence of a close similarity or relation between the two.

Dimensions: Length, 37μ; transdiameter, 34μ; dorso-ventral diameter, 32μ.

Loc. Off Futagojima, Aug. 23, 1926.

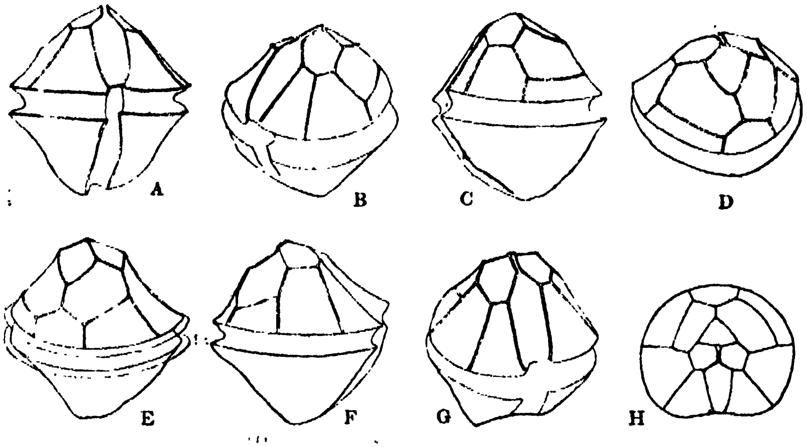


Fig. 32. *Peridinium levanderi*, n. sp.

A, Ventral view. B, Left ventral view. C, Left side view. D, Left dorsal view. E, Right dorsal view. F, Right side view. G, Right ventral view. H, Arrangement of the plates in the epitheca. (×600)

30) **Peridinium munobis**, n. sp.

A small, *achromaticum*-like species with a pre-median girdle. The epitheca exceeds the hypotheca.

The epitheca is flat and conical, with somewhat convex sides and a pointed apex. The apical pore bears a short, narrow ventral slit (but not having any real opening). The plates are of the *Orthoperidinium* type with three dorsal intercalaries. The girdle is relatively wide and circular, bearing the side ridges of the thecal wall. The furrow is deeply impressed with numerous transverse striations on its wall. The ventral area is wide, guarded by very low side fins increasing its width posteriorly, of which the posterior parts will be easily misinterpreted as minute antapical spines in a ventral view. The hypotheca is rounded posteriorly, with a swollen, distinctly convex, ventral side; its posterior end is flattended dorso-ventrally. The ventral area is wider than the girdle of which the posterior end is slightly indented.

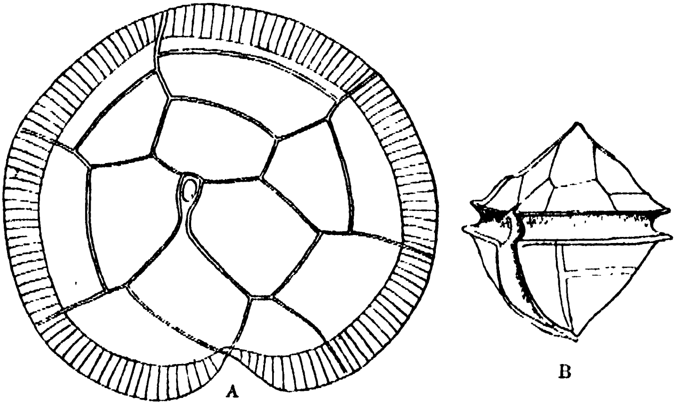


Fig. 33. *Peridinium munobis*, n. sp.
A, Apical view. (×1600) B, Left side view. (×600)

In inspecting plankton this species is apt to be easily confused with *P. achromaticum* and *P. levanderi* because of their minute size and broad, rhombic shape. This species is distinguished at once from them by its anteriorly displaced girdle plane. *P. achromaticum* is easily distinguished from *P. levanderi* by its larger size, relatively pointed antapical end and somewhat wider ventral area. In the three, the precingular series of plates of this species are the least developed in contrast to the well developed apical plates.

Dimensions: Length, 44μ; transdiameter, 39μ.
Loc. Off Futagojima, Aug. 23, 1926.

31) **Peridinium biconicum,** n. sp.

A medium-sized, somewhat elongated, rhombic species with a hyaline antapical fin and a descending girdle.

The body is biconical antero-posteriorly with straight or slightly concave sides. The epitheca is conical with a slit-like apical pore at its pointed apex. The pore has long ventral and dorsal slits, resulting in an elongated aperture with a somewhat wider part, the apical pore, near its dorsal end. The lateral walls of the pore project in short finger-like processes at the anterior ends of the intercalary bands between the apical plates *2–3*, and *3–4*. In some specimens a flat, hyalious, membrane-like structure is seen along the whole length of the aperture, but it probably is a process of the inner plasm through the pore. The plate formula is $4'$, 2^{γ}, 7^{a} and 5^{A}, 2^{l}. The apical *1* is a long, narrow plate of which the surface is slightly concave, and the dorsal rhombic *3* is smaller than the lateral ones. The ventral precingulars *a* and *g* are the largest and the dorsal pentagonal *d* is the smallest in the series. The large hexagonal intercalaries γ and ε are in the middle of the dorsal surface bilaterally, in saddle fashion. The intercalary bands are broad and well developed, with transverse striations.

The girdle is median, slightly descending, and is displaced distally 1.5 girdle width. The furrow is deeply impressed with side lists and numerous transverse parallel ribs and grooves on its wall. The ventral area is narrow but is deeply impressed, without posterior widening and with low lateral fins. The ventral area extends to the post-margin, diminishing in depth, with a deep subterminal, asymmetrical excavation on the ventral left side, so that at the hindermost part of the body, the left half of the ventral part is impressed, making the remaining part somewhat obliquely semi-circular in cross section, while the post-margin forms a small shovel-like fin. In some specimens a spine-like thickening is seen medially. The ventral side of the hypotheca is strongly convex, and along its median line is the deeply excavated ventral area. The surface is smooth without any markings.

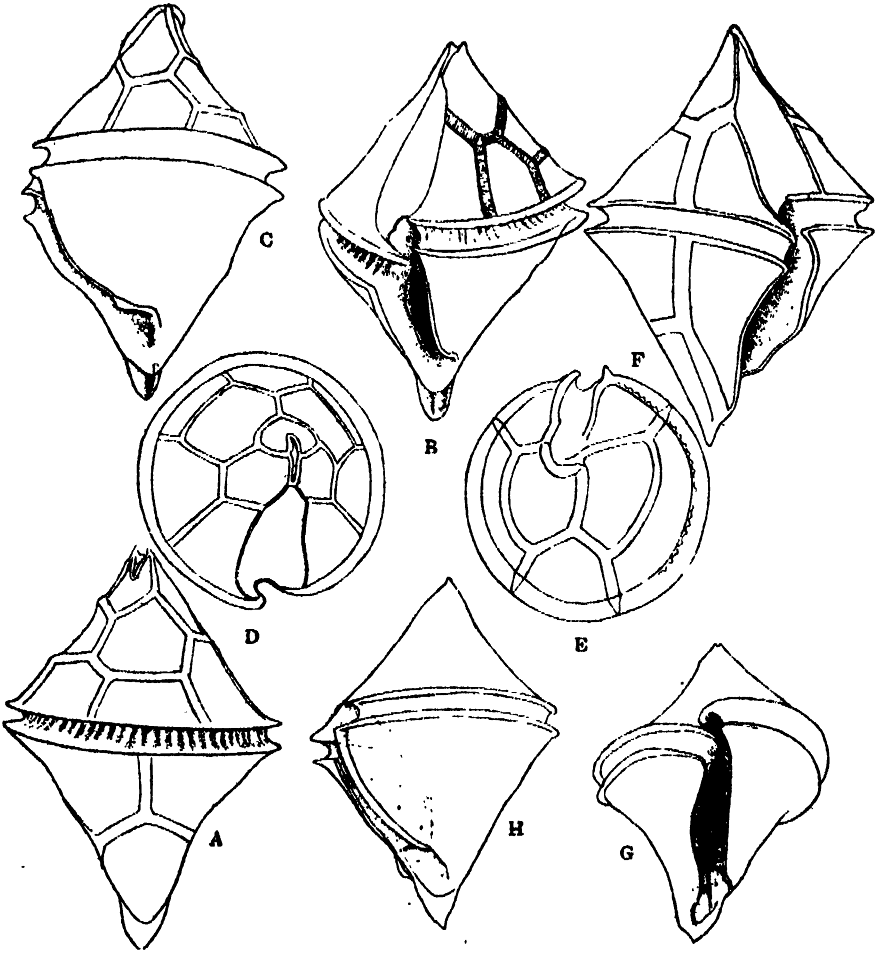


Fig. 34. *Peridinium biconicum*, n. sp.

A, Dorso-lateral view. B, Ventral view. C, Left side view. D and E, Arrangement of the plates on the epitheca and the hypotheca. F, Right ventral view of another specimen. G, Antapical ventral view of the hypotheca, showing the ventral area of a smaller specimen. H. Left side view of G specimen showing the depression of the ventral area, the flagellar pore, and the side fins of the furrow. (× 600)

Murrayella punctata (CLEVE), *Heterocapsa triquetra* STEIN, and *Ceratium biconicum* MURR. and WHITT. are closely resembling species. *Murrayella punctata* differs from this species in having distinct surface markings, a rounded anterior end, short, oblique, ventral area and no antapical fin. This also differs from *Heterocapsa triquetra* in size and in having a more pointed apical end, a narrower girdle, a less developed antapical horn, no ventral epithecal furrow, and a straight but long posterior-ventral furrow. From *Ceratium biconicum* it differs profoundly in its broader body, pointed anterior end and faintly listed, deep, ventral furrow.

Dimensions: Length, 70μ; transdiameter, 47μ; dorso-ventral dimension, 44μ.

Loc. Off Futagojima, Aug. 20, 1926.

32) **Peridinium ventricum,** n. sp.

A small, asymmetrically biconical species. The hypotheca markedly exceeds the epitheca.

The epitheca is a flat subcone, bluntly pointed anteriorly, with a slight ventral concavity. The apical pore is not on the anterior extremity but is distinctly displaced ventrally, lying in the node of the apical plates *1*, *2*, and *4*, bearing a peculiarly long dorsal slit (truly only a groove) instead of a ventral one, passing over the anterior end, extending to the middle of the dorsal apical plate *3*. The apical pore is in the ventralmost end of the slit. The plates are of the *Orthoperidinium* type with two unequal intercalaries. The dorsal apical plate *3*, the intercalaries, and the precingular plate *d* are displaced somewhat to the right side of the body and all of them are more or less irregularly shaped. The section at the girdle is semicircular with a slight ventral excavation. The girdle is relatively wide, deeply impressed, descending, and is displaced distally one girdle width, with side-ridges of the thecal wall. The ventral area is narrow and deeply impressed, extending to the antapex where it widens slightly. The hypotheca is a somewhat elongated subcone with concave dorsal and somewhat convex ventral sides, and narrow but faint post-indentation, bearing or not bearing a low fin along its right post-margin. The postcingulars are one-third of the antapicals in their altitude except the ventral *A* and *E* which occupy its entire length. The surface is smooth and the intercalary bands are very narrow.

From *P. biconicum*, which this species resembles most closely, it is distinguished at once by its smaller size and anteriorly displaced girdle plane. This dorsal elongation of the apical slit-like groove is also fairly developed in *P. biconicum* in which the pore lies a little ventrally from its dorsal end. This ventral displacement of the apical pore or the dorsal elongation of the slit of this biconical species serves to separate it from all other species in the genus.

Dimensions: Length, 51-54μ; transdiameter, 42-47μ.

Loc. Off Futagojima, Aug. 23, 1926.

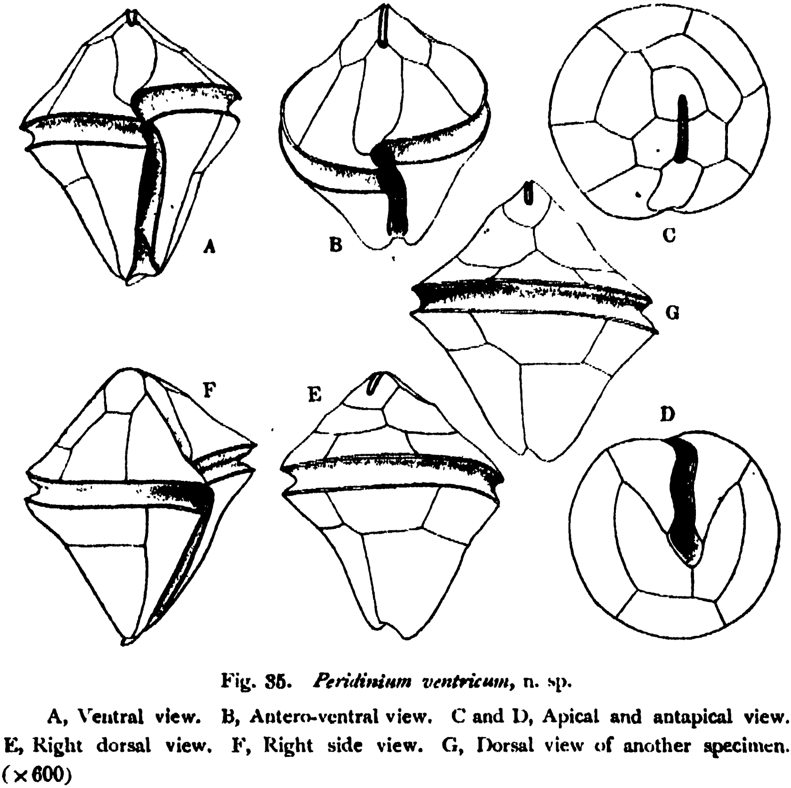


Fig. 35. *Peridinium ventricum*, n. sp.

A, Ventral view. B, Antero-ventral view. C and D, Apical and antapical view. E, Right dorsal view. F, Right side view. G, Dorsal view of another specimen. (×600)

CONGRUENTIDIUM, n. gen.

The body is of nearly bilateral symmetry. In ventral view its form resembles some *Peridinium* in the presence of a small apical horn and widely separated antapical spines. The body is strongly dorso-ventrally flattened, with a dorsally inclined girdle plane. The epitheca is subequal to the hypotheca, Its peripheral part is thin, forming a meridional edge. The plates are as follow: four apicals, one apical intercalary, five precingulars, five girdl plates, five postcingulars, one antapical intercalary, and two antapicals. In the smaller number of the precingular plates and in the development of the intercalary plate in the rear of the ventral area, coupled with the dorso-ventral flattening, it differs from *Peridinium*. By the different number and the symmetrical arrangement of the plates, and by the presence of the nearly completing girdle, it is distinguished from *Heterodinium*.

33) **Congruentidium compressum**, n. sp.

A peculiarly flattened, pentagonal species with a minute apical horn and two small, widely separated antapical spines intervening a shallow but wide post-indentation.

The body is pentagonal, strongly flattened dorso-ventrally, its length is 1.03 transdiameter and its dorso-ventral diameter on the middle of the girdle level is 0.26 transdiameter. The cross-sections at the girdle and in the dorso-ventral meridional plane are both elongated and are somewhat slightly deformed spindle shapes. The epitheca is not so strongly flattened as the hypotheca, as the post-marginal part of the latter is extremely thin. The epitheca is broad and slender conical in ventral and side views, respectively, provided with convex sides and a slight step-like shoulder in the apical-precingular suture. Its sides contract to a small but distinct apical horn, widening slightly anteriorly, with a minute pore and a narrow ventral slit which continues to a fine canal leading into the inner cavity. The thecal wall is thick, especially in the marginal parts where the dorsal and the ventral walls fuse to form a broad and very thick wall except at the apex where a fine canal connects the narrowed cavity with the pore penetrating through the wall. The plate structure is obscured by the inner plasm at the central part, so that it is very difficult to analyse fully the relations of the plates in the central region. The plates comprising the epitheca are 10 in number. The mid-ventral apical *1* is an elongated, rhombic plate narrowing anteriorly, and the other apicals are all triangular, two small ones, *2* and *4*, on the lateral sides of the former and a large one, *3*, on the dorsal, occupying nearly its anterior half with an incurved basal rim. The precingular plates are all low in altitude, numbering 5, comprising two ventral, two side, and one flat dorsal plate. Along the middle of the side precingular plates are the marginal body edges. A flat, somewhat lens-shaped intercalary plate is on the dorsal side, two ends of which are truncated obliquely. The girdle is relatively wide, ascending slightly, and is displaced distally. It is median in the ventral and distinctly post-median in the dorsal, so that the girdle plane inclines dorsally about 60°. The furrow is deeply impressed with low but stout sidelists. The ventral area is narrow and short, only extending posteriorly half way between the girdle and the post-margin. It expands a little abruptly to the right to form a pocket-like depression in the bottom of which lies the flagellar pore, but in some specimens this impression is seen along the middle of the area instead of on its right side. The anterior plate indents the hypotheca a little, ending in a sharply truncated terminal. The hypotheca is broad and trapezoidal with two spines on the widely separated angles and also shoulders in the postcingular-antapical sutures. The post-margin is slightly indented, the bottom of which is nearly straight, and slightly convex or concave at the intra-antapical suture. The antapical horn is short, and is hardly appreciable in some specimens, ending in a small spine. Low hyaline, denticulated fins are seen in some of them, along the sides and the posterior margins of the antapical plates. The postcingular-antapical suture is broad, subparallel to the girdle in the dorsal, but strongly displaced posteriorly in the ventral side by the development of the large ventral intercalary plate. The postcingular plates are 5 comprising one dorsal, two lateral, and two ventral ones. The antapical are two, each covering the postero-lateral corners. A large intercalary plate is at the rear of the ventral area, indenting the antapicals deeply. It is also slightly concave following the ventral area. The intercalary bands between different series of plates are broad and striated, but those of the intraserials are narrow, except the intra-antapical one. The surface is smooth.

This peculiar and interesting species has, as far as I know, clearly defined and isolated characters which distinguish it from any other species of all the *Peridiniales*. It is remarkable in form, in the strong dorso-ventral flattening and in the dorsal inclination of the girdle plane, as well as in the number and the arrangement of the thecal plates.

In the characters of the widely separated antapical spines, it is closely related to *Gonyaulax catenata*, but differs in having the small antapical horn, smaller number of the antapical spines, the epithecal and the hypothecal shoulders, and the strong flattening. From some strongly flattened species of *Heterodinium*, it is distinguished by its plate arrangement and the dorsally inclined girdle plane, as well as in having a complete girdle furrow and two antapical plates.

Dimensions: Length, 62–65μ; transdiameter, 58–63μ; dorso-ventral dimension, 15μ.

Loc. Off Futagojima, Aug. 23, 1926.

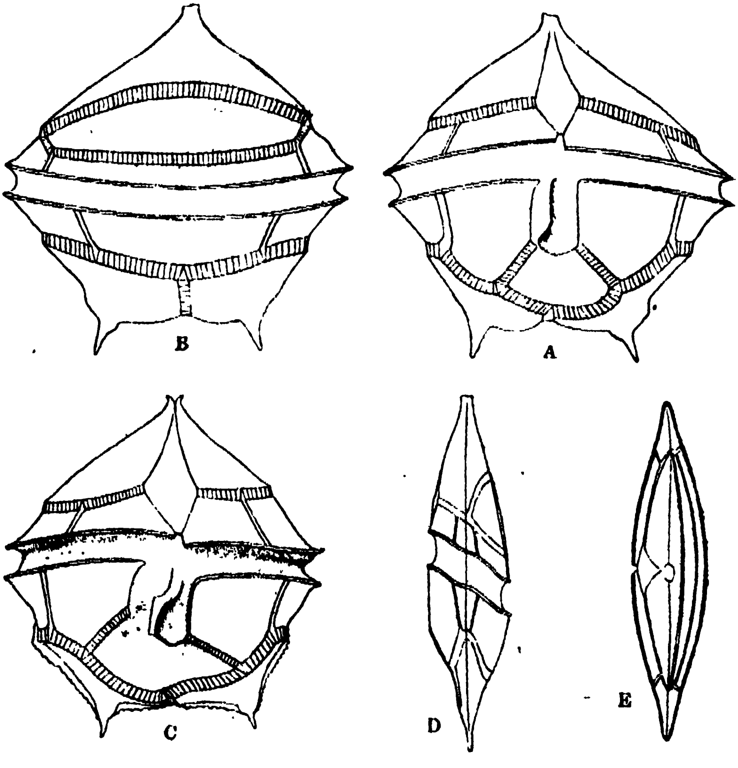


Fig. 36. *Congruentidium compressum*, n. g., n. sp.

A, Ventral view. B, Dorsal view. C, Ventral view of another specimen. D, Side view. E, Diagram of the pattern of the plate in the epitheca. (×600)

CERATIUM Schrank.

34) **Ceratium furca** subsp. **eugrammum** (Ehrbg).

Jørgensen 1911, Pl. II, Figs. 24, 25, 26.
* *Peridinium eugrammum* Ehrenberg 1859, p. 792.

Dimensions: Length, 135μ; transdiameter, 17μ.

Loc. Off Asamushi, Aug. 16, 1626.

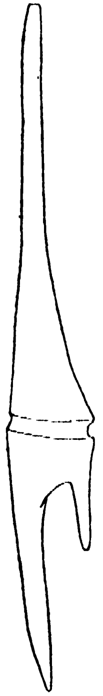


Fig. 37. *Ceratium furca* subsp. *eugrammum* (Ehrbg). (×600)

35) **Ceratium fusus** (Ehrbg).

*Ehrenberg 1833, p. 271, 1834, Pl. II, Fig. 3.
Paulsen 1908, p. 91, Fig. 123.
Okamura & Nishikawa, 1904, p. 127, Figs. 22, 23.
Schütt 1895, Pl. IX. Fig. 35.

The right antapical horn is short and rounded.

Dimensions: Length 400μ; transdiameter, 24μ.

Loc. Off Asamushi, Aug. 10, 1926.

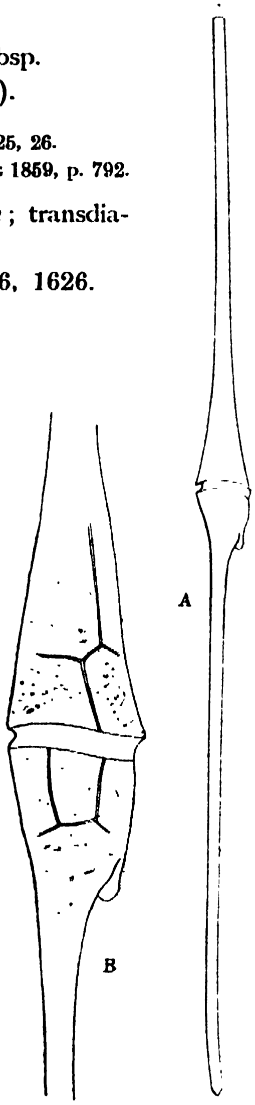


Fig. 38. *Ceratium fusus* (Ehrbg).
A, Straight specimen.(×280)
B, Mid-body. (×600)

36) **Ceratium pulchellum** Shröder.

Schröder 1900, Pl. I, Fig. 17c.
Jørgensen 1911, p. 33, Pl. III, Figs. 59, 60, 61.

Dimensions: Length, 400μ; transdiameter, 85μ.

Loc. Off Asamushi, Aug. 20, 1926.

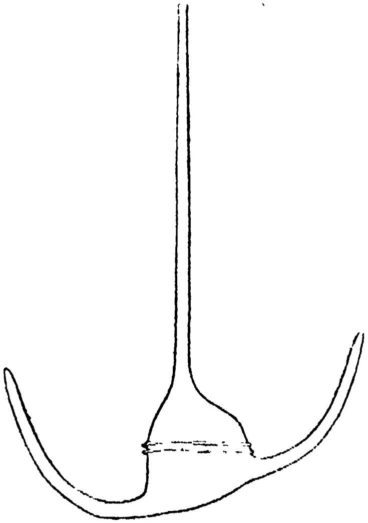


Fig. 39. *Ceratium pulchellum* SCHRÖD. (×280)

37) Ceratium pulchellum var. longipes, n. var.

The shallow post-indentation and widely spreading antapical horns serve to distinguish this variety from the former species.

Dimensions: Length, 430μ; transdiameter, 81μ.

Loc. Off Asamushi, Aug. 23, 1926.

38) Ceratium tripos (O. F. MÜLLER) var. atlantica OSTFLD.

*OSTENFELD 1903, p. 584, Figs. 132, 133.
PAULSEN 1908, p. 77. Fig. 120.
JØRGENSEN 1911, p. 36, Pl. IV, Figs. 69–73.

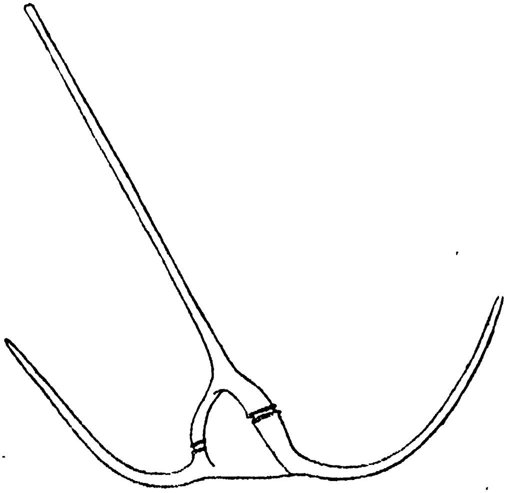


Fig. 40. *Ceratium pulchellum* var. *longipes*, n. var. (×280)

Dimensions: Length, 260–300; transdiameter, 73–78μ.
Loc. Off Asamushi Aug. 8, 1926.

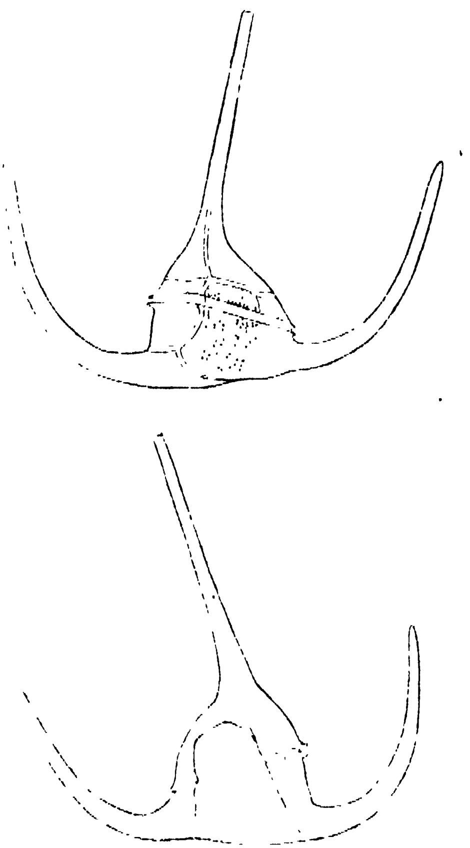


Fig. 41. *Ceratium tripos* var. *atlantica* OSTFLD. (×285)

39) **Ceratium tripos** var. **subsalsum** OSTFLD.

*OSTENFELD 1903, p. 584, Fig. 134.
PAULSEN 1908, p. 79, Fig. 104.
BORGERT 1919, Pl. I.
JØRGENSEN 1911, p. 36, Pl. III, Figs. 66, 67.

Common species in summer.
Dimensions: Length, 94–153μ; transdiameter, 41–49μ.

Loc. Off Asamushi, Aug. 20, 1926.

40) Ceratium bucephalum var. heterocamptum JϕRGENSEN.

C. bucephalum var. *heterocamptum* JϕRG. 1899, p. 44, Pl. II, Fig. 12.

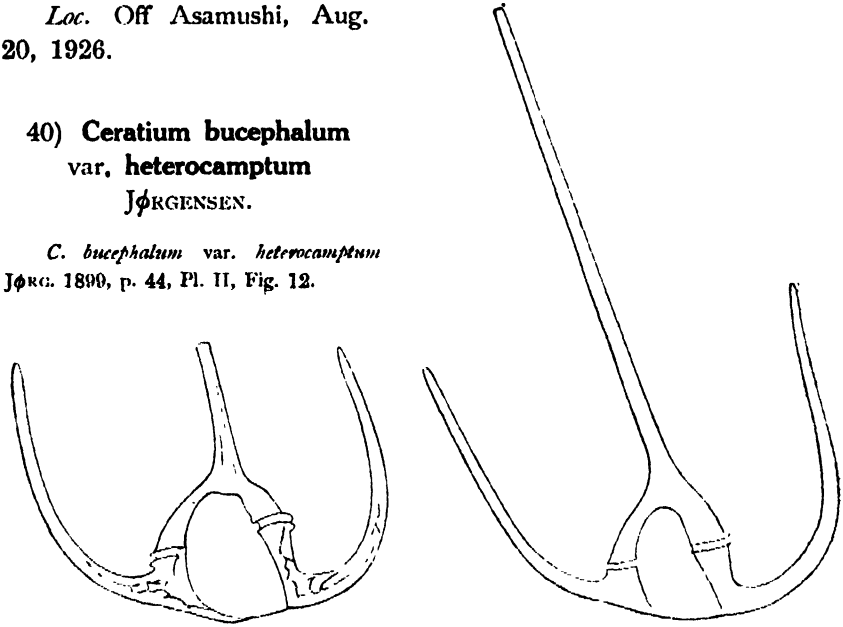


Fig. 42. *Ceratium tripos* var. *subsalsum* OSTFLD. (×280)

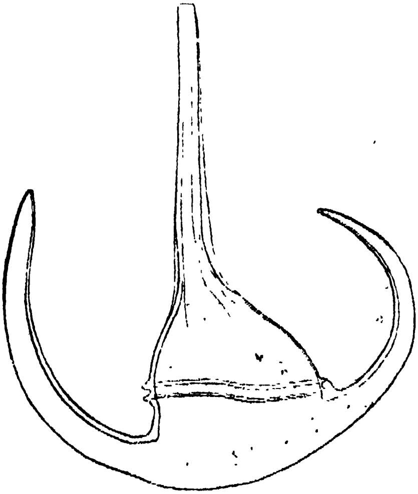


Fig. 43. *Ceratium bucephalum* var. *heterocamptum* JϕRG. (×280)

C. (*tripos* var.) *arietinum* CLEVE 1900 (a), p. 13, Pl. VII, Fig. 3.

C. heterocamptum, PAULSEN 1908, p. 76, Fig. 101.

C. buceph. var. *heterocamp.* JϕRGENSEN 1911, p. 48, Pl. V. Fig. 101.

The distal part of the right antapical horn shows a characteristic curve, distinctly turning inward nearly perpendicular to the apical horn.

Dimensions: Length, 162μ; transdiameter, 56μ.

Loc. Off Asamushi, Aug. 10, 1926.

41) **Ceratium macroceros** (EHRBG).

* EHRENBERG 1840. p. 201.
OKAMURA and NISHIKAWA 1904, p. 122, Fig. 2.
OKAMURA 1907, p. 129, Pl. IV, Fig. 23.
PAULSEN 1908, . 81, Fig. 81.
JØRGENSEN 1911, p. 63, Pl. I, Fig. 6; Pl. VII, Figs. 132, 133.

A thin-walled, delicate species common in the summer plankton at Asamushi.

Dimensions: Length, 430–500μ; transdiameter, 55–60μ.

Loc. Off Asamushi, Aug. 21, 1926.

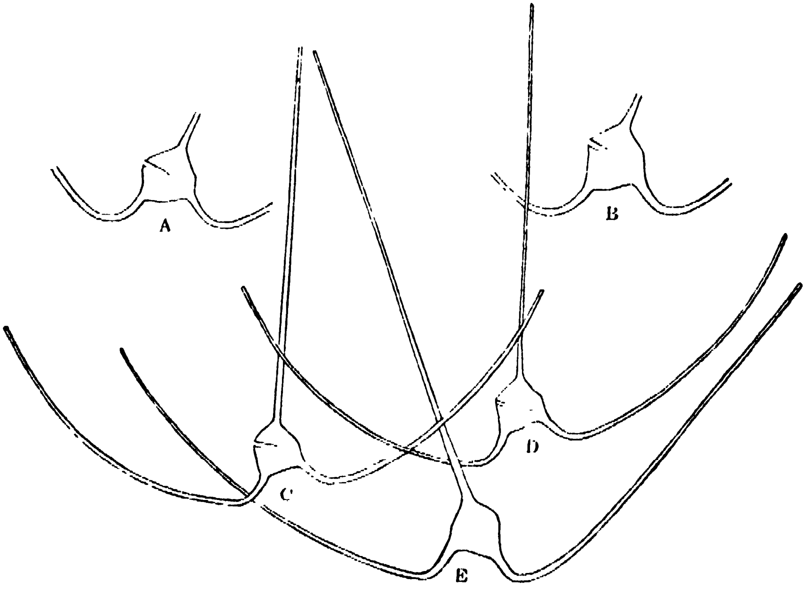


Fig. 44. *Ceratium Macroceros* (EHRBG.).

A, B, C, D. (×120) E, Intermediate form between this and subsp. *gallicum*. (×120)

42) **Ceratium macroceros** (EHRBG). var. **karsteni,** n. var.

C. tripos macro. EHRBG. var. *crassa* KARSTEN 1907, Pl. XLIX, Fig. 27e.

The mid-body is slightly larger than the preceding and is remarkably swollen anteriorly, so that the transdiameter at the girdle is shorter than that of the largest part of the pregirdle and the mid-body is somewhat pentagonal in ventral view.

The basal part of the apical horn is thick and gradually tapers distally. The right antapical horn usually curves with a smaller curvature than the preceding. The post-margin is always narrower than the transdiameter at the girdle. The surface is smooth.

Dimensions; Length, 500μ; transdiameter, 67μ.

Loc. Off Asamushi, Aug. 10, 1926.

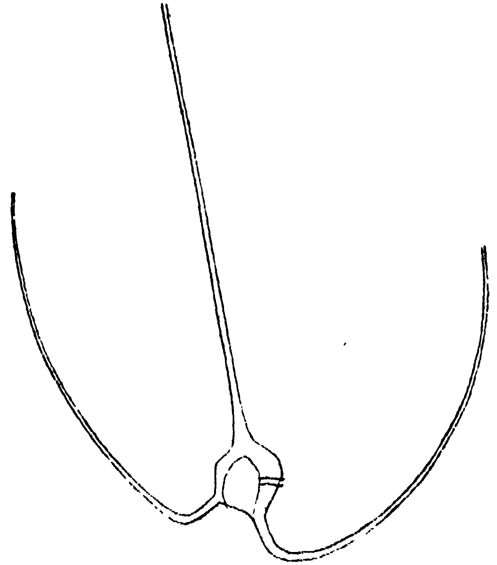


Fig. 45. *Ceratium macroceros* (EHRBG). CLEVE var. *karsteni*, n. var. (×120)

43) Ceratium macroceros (EHRBG.) forma (?)

The post-indentation is much deeper than that of *C. Carriense* f. *ceylanicum* (B. SCHR.) *Jörg.* and *C. tenue* var. *buceros* (ZACHARIAS). Also the girdle lists are complete. The deep post-indentation and the mid-body closely correspond to *C. macroceros.*

Dimensions: Length, 350μ; transdiameter, 55μ.

Loc. Off Asamushi, Aug. 25, 1926.

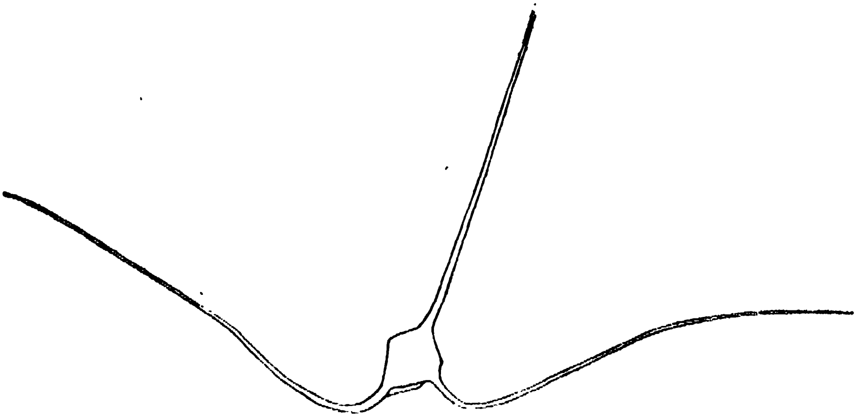


Fig. 46. *Ceratium macroceros* (EHRBG.) forma (?) (×120)

44) **Ceratium macroceros** subsp. **gallicum** (KOFOID).

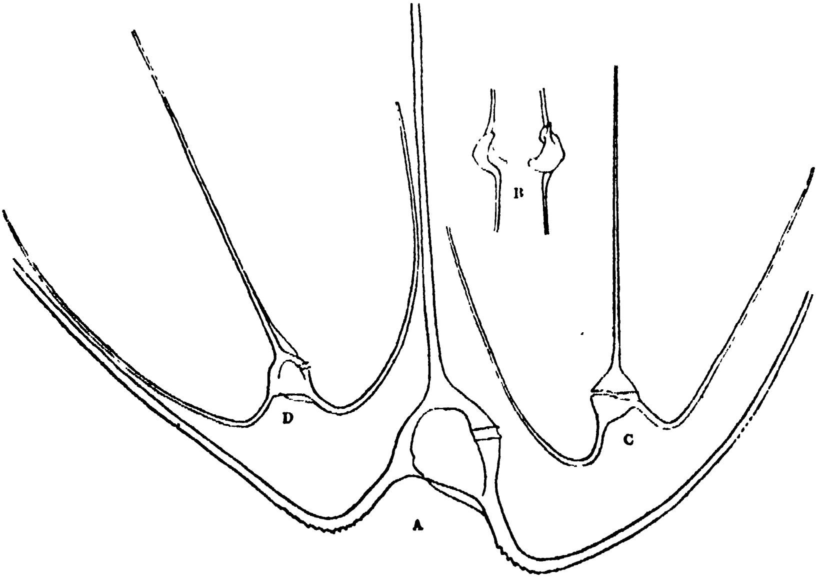


Fig. 47. *Ceratium macroceros* subsp. *gallicum* (KOFOID).

A, (×300) B, Apical and antapical view of mid-body. (×120) C, (×120) D, A form transitional to *C. macroceros* species. (×120)

Kofoid 1907, p. 302, Pl. XXIV, Figs. 10–12.
Jørgensen 1911, p. 63, Pl. VII, Figs. 134, 135.
Forti 1922, p. 63, Pl. III, Fig 48.

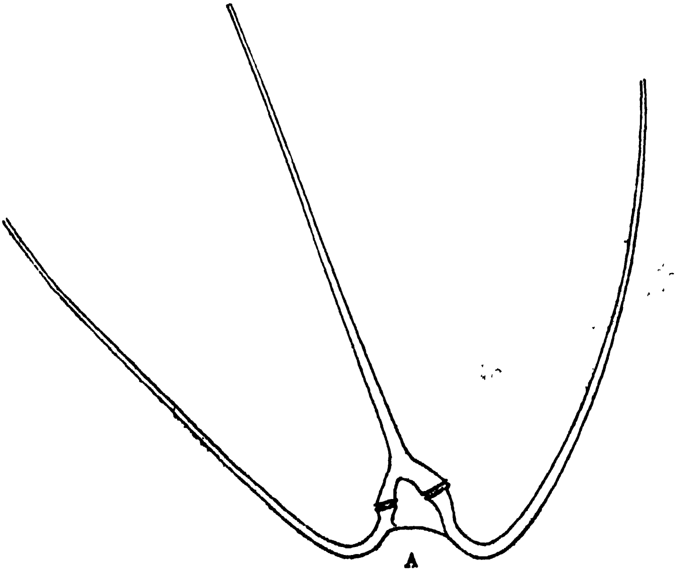
This is distinguished from *C. macroceros* mainly by its right antapical horn, sharply bent at a little distance from its proximal end, somewhat intervening a straight part. The mid-body is small with a straight post-margin. There seems to be a tendency, as measurements show, that the narrower the proximal antapical angle, the longer the proximal straight part of the right antapical horn.

Dimensions: Length, 470–615μ; transdiameter, 50–68μ.

Loc. Off Asamushi, Aug. 8, 1926.

45) **Ceratium massiliense** (Gourret).

Jørgensen 1911, p. 66, Pl. VII, Figs. 140, 141, 142.
Okamura 1912, p. 9, Pl. I, Fig. 13.
C. trip. var. *massiliense* Gourr. 1883, p. 27, Pl. I, Fig. 2.
C. trip. macroceroides Karsten 1906, Pl. XXII, Fig. 28 a. b.
C. trip. macroceros Karsten 1906, Pl. XXII, Fig. 29 a.
C. ostenfeldii Kofoid 1907(e), p. 305, Pl. XXVI, Fig. 22–25.



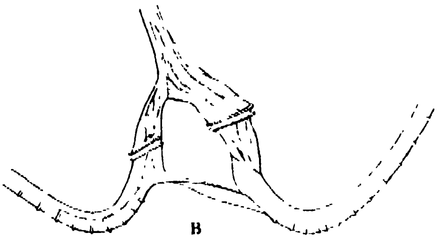


Fig. 48. *Ceratium massiliense* (GOURRET).
A. (×120) B, Ventral view of mid-body. (×280)

A stout, spreading species common in late summer plankton at Asamushi, with a thick wall and a wrinkled surface.

Dimensions: Length, 550μ; transdiameter 75μ.

Loc. Off Asamushi, Aug. 20, 1926.

46) Ceratium trichoceros (EHRBG).

KOFOID 1908(b), p. 388.
JØRGENSEN 1911, p. 75, Pl. IX. Fig. 159.
* *Peridinium trichoceros* EHRENBERG 1859, p. 791, 1873, p. 3, Fig. I.
C. flagelliferum CLEVE(a) 1900, p. 14, Pl. VII, Fig. 12.
C. tripos flagelliferum KARSTEN 1906, Pl. XXII, Fig. 31b.
C. tripos flagell. forma *crasses* KARSTEN 1906, Pl. XXII, Fig. 32 a, b.

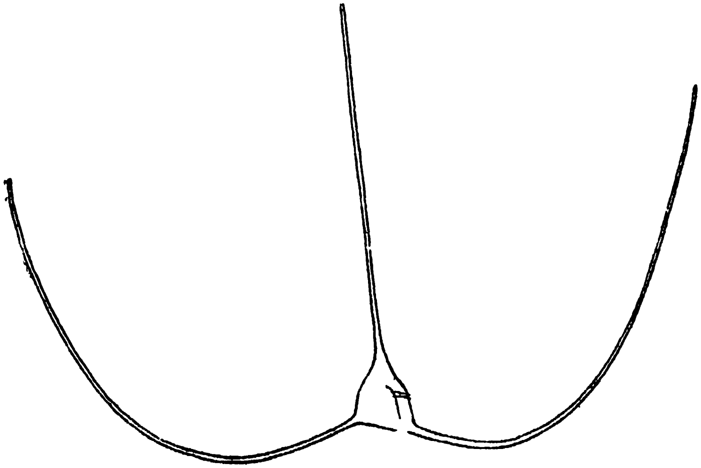


Fig. 49. *Ceratium trichoceros* (EHRBG). (×120)

The mid-body is tall and triangular and little smaller than that of *C. inflexum*. The basal part of the left antapical horn is delimited from the post-margin.

Dimensions: Length, 400μ; transdiameter, 54μ.

Loc. Off Asamushi, Aug. 24, 1926.

47) **Ceratium inflexum** (GOURRET).

KOFOID 1908(b), p. 388.
JØRGENSEN 1911, p. 76, Pl. IX, Figs. 160, 161.
C. tripos var. *typicum* GOURRET 1883, p. 31, Pl. II, Fig. 36.
C. flagelliferum var. *filiformis* OKAMURA et NISHIKAWA 1904, p. 123, Pl. VI, Fig. 11.
C. tripos flagell. forma *angulata* KARSTEN 1906, Pl. XXII, Fig. 33.
C. trip. var. *macroceras* forma *undulata* KARSTEN 1907, Pl. XLVII, Fig. 28.

This is distinguished from *C. trichoceros* by its larger, but flat and triangular mid-body.

Dimensions: Length, 350–420μ; transdiameter, 54–63μ.

Loc. Off Asamushi, Aug. 24, 1926.

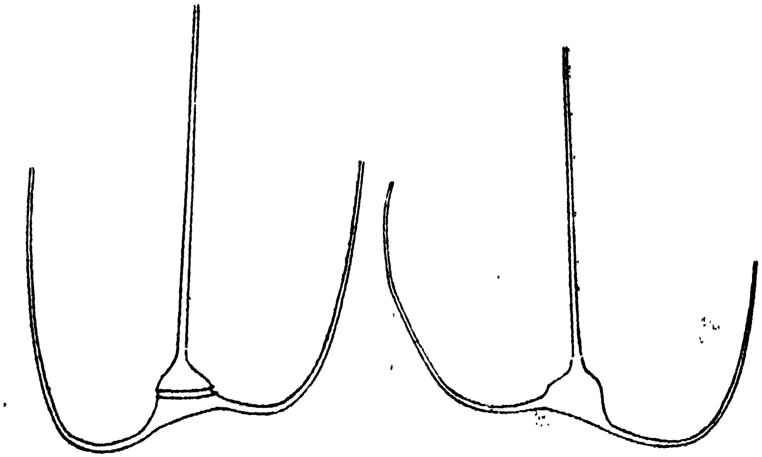


Fig. 50. *Ceratium inflexum* (GOURRET). (×120)

LITERATURES

1) ALLEN, W. E., 1922. Quantitative studies on inshore marine Diatoms and Dinoflagellates of Southern California in 1920. Univ. Calif. Publ. Zool. XXII.

2) ALLEN, W. E., 1923. Studies on marine Diatoms and Dinoflagellates caught by aid of the Kofoid bucket in 1922. Univ. Calif. Publ. Zool. XXII.

3) APSTEIN, C., 1905. Plankton in Nord-, und Ostsee auf den deutschen Termenfahrten. 1. Teil. Wissensch. Meeresuntersuch. Abt. Kiel. IX.

4) APSTEIN, C., 1906. *Pyrocystis lunula* und ihre Fortpflanzung. Wissensch. Meeresuntersuch. Abt. Kiel. IX.

5) APSTEIN, C., 1910. Biologische Studie über *Ceratium tripos* var. *subsalsa* OSTFLD. Wissensch. Meeresuntersuch. Abt. Kiel. XII.

6) AURIVILLIUS, C. W. S., 1898. Vergleichende tiergeographische Untersuchungen über die Plankton-Fauna des Skageraks in den Jahren 1893–1897. K. Svensk. Akad. Handl. XXX.

7) AURIVILLIUS, C. W. S., 1899. Animalisches Plankton aus dem Meere zwischen Jan Mayer, Spitzbergen, K. Karls Land und der Nordküste Norwegens. K. Svensk. Akad. Handl. XXXII.

8) BARROWS, A. L., 1918. The significance of Skeletal variations in the genus *Peridinium*. Univ. Calif. Publ. Zool. XIIX.

9) BERGH, R. S., 1881. Der Organismus der Cilioflagellaten. Journ. Morph. VII.

10) BERGH, R. S., 1882. Über die systematische Stellung der Gattung *Amphidinium*. Zool. Anz. V.

11) BERGH, R. S., 1887. Über den Teilungsvorgang bei den Dinoflagellaten.' Zool. Jahrb. II.

12) BORGERT, A., 1910. Kern- und Zellteilung bei marinen *Ceratium*-Arten. Arch. f. Protistk. XX.

13) BROCH, HJ., 1906. Bemerkungen über den Formenkreis von *Peridinium depressum* S. lat. Nyt Magazin for Naturvidensk. LIV.

14) BROCH, HJ., 1910. Die *Peridinium*-Arten des Nordhafens (Val de Bora) bei Rovigno in Jahre 1909. Arch. f. Protistk. XX.

15) BUTCHINSKY, P., 1897. Die Protozoen-Fauna der Salzsee-Limane bei Odessa. Zool. Anz. XX.

16) BÜTSCHLI, O., 1873. Einige über Infusorien. Arch. f. Mikrosk. Anat. IX.

17) BÜTSCHLI, O., 1885. Einige Bemerkungen über gewisse Organisationsverhältnisse der sog. Cillioflagellaten u. der *Noctiluca*. Morph. Jahrb. X.

18) CALKINS, G. N., 1899. Mitosis in *Noctiluca* and its bearing on the nuclear relations of the Protozoa and the metazoa. Journ. Morph. XV.

19) CARTER, H. J., 1863. Notes on the colouring matter of the Red Sea. Ann. and Mag. of Nat. Hist. Ser. 3. XI.

20) CARTER, H. J., 1871. Notes on a Freshwater Species of *Ceratium* from the Lake of Nynee (naini) Tai in Kumaon. Ann. and Mag. of Nat. Hist. Ser. 4. VII.

21) CLEVE, P. T., 1900(a). Notes on some Atlantic Plancton Organisms. K. Svensk. Akad. Handl. XXXIV.

22) CLEVE, P. T., 1900(b). The Plancton of the North Sea, the English channel and the Skagerak in 1899. K. Svensk. Akad. Handl. XXXII.

23) Cleve, P. T., 1901 (a). Plancton from the southern Atlantic and the southern Indian Ocean. Öfvers. af. K. Svensk. Akad. Förhandl. IIX.

24) Cleve, P. T., 1901 (b). Plankton from the Red Sea. Öfvërsigt af. K. Vetensks. Akad. Förhandl. IX.

25) Cleve, P. T., 1901 (c). The seasonal distribution of Atlantic Plankton Organisms. Göteborgs K. Vetensk. och. Vitterhet. Handl. XVII.

26) Cleve, P. T., 1902. The plankton of the North Sea and the Skagerats in 1900. K. Svensk. Akad. Handl. XXXVI.

27) Conrad, W., 1926. Recherches sur les Flagellates de nos eaux saumatres. Ie Partie: Dinoflagellates. Arch. f. Protistk. XLV.

28) Dogiel, V., 1906. Beiträge zur Kenntnis der Peridineen. Mitt. Zool. Stat. Neapel. XIIX.

29) Driver, H., 1907. Das Ostseeplankton der 4 deutschen Terminfahrten im Jahre 1905. Wissensch. Meeresuntersuch. Abt. Kiel. X.

30) Entz, G., 1926. Beiträge zur Kenntnis der Peridineen. Arch. f. Protistk. LVI. p. 397.

31) Fauré-Fremiet, E., 1908. Étude descriptive des Peridiniens et des infusoires cilies du plankton de la Baire de la Hougue. Ann. d. Sci. Nat. (Zool.), Sér. 9. VII.

32) Forti, A., 1922. Recherche su la floro pelagica (fitoplancton) di Quarto dei Mille. Lab. marin. d. Quarto d. Mille pr. genova. Mem. XCVII.

33) Frenzel, T., 1891. Über einige merkwürdige Protozoen Arzentiniens. Zeitschr. f. wiss. Zool. LIII.

34) Geither, L., 1926. Über Chromatophoren und Pyrenoide bei Peridineer. Arch. f. Protistk. LIII.

35) Gourret, P., 1883. Sur les péridiniens du golfe de Marseille. Ann. Mus. d'hist. nat. Marseille Zool., I, No. 8.

36) Hirasaka, K., 1922. On a case of discoloured Sea water. Annot. Zool. Japons. X.

37) Imhof, O. E., 1883. Resultate einer Studien über die pelagische Fauna d. Süsswasserbecken der Schweiz. Zeits. f. Wiss. Zool. XL.

38) Jollos, V., 1910. Dinoflagellatenstudien. Arch. f. Protistk. XIX.

39) Jørgensen, E., 1899. Protophyten u. Protozoen in Plankton aus der norwegische Westküste. Berg. Mus. Aarb. VI.

40) Jørgensen, E., 1900. Protistenplankton aus dem Nordmeere in den Jahren 1897–1900. Berg. Mus. Aarb. VI.

41) Jørgensen, E., 1911. Die Ceratien. Eine kurze Monographie der Gattung *Ceratium* Schrank. Leipzig.

42) Jørgensen, E., 1912. Bericht über die von der schwedischen hydrographischen biologische Kommission in der schwedischen Gewässern in den Jahren 1909–1910 ein gesammelten plankton Problem. Skr. Schw. Hydro-biol. Komm. 4. 1–20.

43) Karsten, G., 1905. Das Phytoplankton des antarktischen Meeres nach dem Material der deutschen Tiefsee-Expedition 1898–1899. Wissensch. Ergebn. d. deutsch. Tiefsee Exped. II. 1–136.

44) Karsten, G., 1906. Das Phytoplankton des atlantischen Ozeans nach dem Material der deutschen Tiefsee Exped. Wissensch. Ergebn. d. deutsch. Tiefsee Exped. II. 137–219.

45) Karsten, G., 1907. Das Indischen Phytoplankton. Wissensch. Ergebn. d. deutsch Tiefsee Exped. II. 220–548.

46) Klebs, G., 1884. Ein kleiner Beitrag zur Kenntnis d. Peridineen. Bot. Zeit. XLII.

47) Klebs, G., 1885. Über die Organization u. d. systemat. Stellung der Peridineen. Biol. Centralbl. IV.

48) Kofoid, C. A., 1906(a). Dinoflagellata of the San Diego region I. On *Heterodinium*, a new genus of the Peridinidae. Univ. Calif. Publ. Zool. II.

49) Kofoid, C. A., 1906(b). On the significance of the asymmetry in *Triposolenia*. Univ. Calif. Publ. Zool. III.

50) Kofoid, C. A., 1906(c). On the structure of *Gonyaulax triacantha* Jørg. Zool. Anz. XXX.

51) Kofoid, C. A., 1906(d). A discussion of species characters in *Triposolenia*. I. The nature of species charcter. II. The adaptive significance of species characters. III. The coincident distribution of related species. Univ. Calif. Publ. Zool. III.

52) Kofoid, C. A., 1907(a). Reports on the scientific results of the Expedition to the Eastern Tropical Pacific, by the U. S. Fish Comm. Steamer "Albatross." IX. New Species of Dinoflagellates. Bull. Mus. Comp. Zool. at Harvard. L.

53) Kofoid, C. A., 1907(b). On *Ceratium eugrammum* and its related species. Zool. Anz. XXXII.

54) Kofoid, C. A., 1907(c). The plates of *Ceratium* with a note on the unity of the genus. Zool. Anz. XXXII.

55) Kofoid, C. A., 1907(d). Brandenburgische Algen IV. *Gonyaulax palustris* Lemm, eine neue Süsswasser Peridinee. Bot. Centralbl. XXI.

56) Kofoid, C. A., 1907(e). Dinoflagellata of San Diego region III. Description of new species. Univ. Calif. Publ. Zool. III.

57) Kofoid, C. A., 1908(a). Exuviation, autotomy, and regeneration in *Ceratium*. Univ. Calif. Publ. Zool. IV.

58) Kofoid, C. A., 1908(b). Note on some obscure species of *Ceratium*. Univ. Calif. Publ. Zool. IV.

59) Kofoid, C. A., 1909(a). XX. Mutations in Ceratium. Bull. Mus. Comp. Zool. at Harvard. LII.

60) Kofoid, C. A., 1909(b). On *Peridinium steini* Jørgensen, with a note on the nomenclature of the skeleton of the Peridinidae. Arch. f. Protistk. XVI.

61) Kofoid, C. A., 1909(c). The morphology of the skeleton of *Podolampas*. Arch. f. Protistk. XVI.

62) Kofoid, C. A., 1910. A revision of the genus *Ceratocorys* based on skeletal morphology. Univ. Calif. Publ. Zool. VI.

63) Kofoid, C. A., 1911(a). Dinoflagellata of the San Diego region, IV. The genus *Gonyaulax*, with notes on its skeletal morphology and a discussion of its generic and specific characters. Univ. Calif. Publ. Zool. VIII.

64) Kofoid, C. A., 1911(b). On the skeletal morphology of *Gonyaulax catenata* (Levander). Univ. Calif. Publ. Zool. VIII.

65) Kofoid, C. A., 1911(c). Dinoflagellata of the San Diego regson, V. On *Spiraulax*, a new genus of the Peridinida. Univ. Calif. Publ. Zool. VIII.

66) Kofoid, C. A., 1926. On *Oxyphysis oxytoxoides* gen. nov., sp. nov. A dinophysoid

dinoflagellate covergent toward the peridinoid type. Univ. Calif. Publ. Zool. XXVIII.

67) KOFOID, C. A., and MICHENER, JOSEPHINE R., 1912. On the structure and relationships of *Dinosphaera palustris* (LEMM.) Univ. Calif. Publ. Zool. XI.

68) KOFOID, C. A., and SWEZY, 1921. The free living unarmoured Dinoflagellates. Mem. Univ. Calif. V.

69) LEFÈVRE, M. ·Contribution à la flore des Peridiniens de France. Revue Algologique II.

70) LEMMERMANN, E., 1900. Beiträge zur Kenntnis der Planktonalge, VIII. Peridiniales aquae dulcis et submarinae. Hedwigia, XXXIX. Beibl. No. 4.

71) LEMMERMANN, E., 1906. Über das Vorkommen von Süsswasser Formen in Phytoplankton des Meeres. Arch. Hydro-biol. Plankt. I.

72) LEMMERMANN, E., 1907. *Gonyaulax palustris* LEMM. eine neue Süsswasser Peridinee. Beihefte z. Bot. Centralbl. XXI.

73) LEMMERMANN, E., 1910. Beiträge z. Kenntnis d. Planktonalgen, XXX. *Peridinium trochoidum* (STEIN) LEMM. Arch. Hydro-biol. Plankt. V.

74) LEVANDER, K. M., 1892. Notiz über die Täfelung der Schalenmembran des *Glenodinium cinctum* EHRBG. Zool. Anz. No. 405

75) LINDEMANN, E., 1923(a). Neue oder wenig bekannte Protisten. IX. Neue oder wenig bekannte Flagellaten. VIII. I. Neue von G. J. PLAYFAIR beschriebene Süsswasserperidineen aus Australien, mit kritischen Bemerkungen über ihre systematische Stellung. Arch. f. Protistk. XLVII. p. 109.

76) LINDEMANN, E., 1923(b). 2. LEBOUR, M. V., D. Sc., Plymouth: Peridinians. I. *Diplopsalis lenticula* and its relatives. Arch. f. Protistk. XLVII, p. 131.

77) LINDEMANN, E., 1924. Neue oder wenig bekannte Protisten. X. Neue oder wenig bekannte Flagellaten. IX. Mitteilung über nicht genügend bekannte Peridineen. Arch. f. Protistk. XLVII. p. 431.

78) LOHMANN, H., 1908. Untersuchungen zur Feststellung des vollständigen Gehalts des Meeres an Plankton. Wissensch. Meeresuntersuch. Abt. Kiel. X.

79) MANGIN, O., 1911. Modifications de la curasee chez quelques peridiniens. Note preliminaire. Int. Rev. Hydrobiol. IV.

80) MANGIN, O., 1913. Sur la Flore Planctonique de la Rade de Saint-Vaast-La-Hougue 1908–1912. Mouvell. Arch. d. Museum d'Histoire Natur. Ser. 4.

81) MARUKAWA, H.. 1921(a). Plankton List and some new species of Copepods from the northern Waters of Japan. Bull. de L'inst. Océanographique, No. 384.

82) MARUKAWA, H., 1921(b). Explanatory diagrams of the Planctonorganisms, II. Peridineae (Japanese). Tokyo.

83) MINKIEWIEZ, R., 1900. Note sur le saison-dimorphisme chez le *Ceratium furca* DUJ. de la mornoire. Zool. Anz. XXIII.

84) MURRAY, G. and WHITTING, F. G., 1899. New Peridiniaceae from the Atlantic. Trans. o. t. Linnean Soc. of London. Ser. 2. Botany V.

85) NISHIKAWA, T., 1901. *Gonyaulax* and the discolored water in the Bay of Agu. Annot. Zool. Japon. IV.

86) OKAMURA, K. and NISHIKAWA, T., 1904. A list of *Ceratium* in Japan. Annot. Zool. Japon. V.

87) OKAMURA, K., 1907. An Annotated list of plancton microrganisms of the Japanese

coast. Annot. Zool. Japon. VI.

88) Okamura, K., 1912. Plancton organisms from Bonito fishing ground. Rep. Imp. Jap. Bur. Fish., Sci. Invest., I.

89) Ostenfeld, C. H., 1906. Plankton végétal. Catalogue des espècies de plantes et d'animaux observées dans le plankton recueilli pendant les expeditions périodiques depuis le mois d'août 1902 jusqu'an mois de mai 1905. Cons. perman. internat. pour l'explarat. d. l. mer. Publ. d. circomst., XXXIII.

90) Ostenfeld, C. H., 1907. Beiträge zur Kenntnis der Algenflora des Kossozol-Bechens in der nordwestlichen Mongolei, mit spezieller Berücksichtigung des Phytoplanktons. Hedwigia, XLVI.

91) Ostenfeld, C. H., 1908. The Phytoplancton of the Aral Sea and its affluents, with an enumeration of the algae observed. Wissensch. Result. d. Aral Sea Exped. Lief. VIII.

92) Ostenfeld, C. H., 1909(a). Notes on the Phytoplancton of Victoria Nyanza, East Africa. Bull. Mus. Comp. Zool. at Harvard. LII.

93) Ostenfeld, C. H., 1909(b). Catalogs des espices de plantes et dánimaux observess dans le plankton recueille pedant les expeditions peridique depuis le mois dáout jusqu'au moi de mai 1908. Publ. Circ. Cons. Perm. Inst. Expl. de la Mer. No. XLVIII.

94) Ostenfeld, C. H., 1910. Marine plankton from the East-greenland Sea. I. List of diatoms and flagellates. Danmark-ekspeditioentil Grönlands nordöstkyst 1906–1908 III. Nr. II.

95) Ostenfeld, C. H., 1913. De Danske forvandes plankton i Aarene 1898–1901. Phytoplankton og Protozoer. Vid. Sekk. Skr., II.

96) Ostenfeld, C. H., 1915. A list of Phytoplankton from the strait Celebes. Dansk Botanisk Archiv II.

97) Ostenfeld, C. H., 1916. Catalogue des Especies de plantesct d'animaux observées dans le plankton recueilli pedant les expédition depuis le mois de juillet 1908 jusqu'au mois de december 1911. Publ. Circ. Cons. Perm. Int. Expl. de la Mer., No. LXX.

98) Ostenfeld, C. H., and Paulsen, O., 1911. Marine plankton from the East-Greenland Sea. IV. General Remarks on the Microplankton. Danmark ekspeditioentil Grönlands nordöstkyst. 1906–1908. III. No. 11.

99) Ostwald, Wo., 1902. Zur Theorie des Planktons. Biol. Centralbl XXII.

100) Ostwald, Wo., 1903. Theoretische Planktonstudien. Zool. Jahrb., Abt. Syst. XVIII.

101) Pascher, A., 1922. Neue oder wenig bekannte Protisten. IV. Neue oder wenig bekannte Flagellaten. IV. Neuebeschriebene Dinoflagellaten bei Jadwiga Woloszynska (Polnische Süsswasserperidineen. Bull. Acad. d. Sciences de Cracovie. Class. Math. Nat. Ser. B: Scienc. Nat. 1916). Arch. f. Protistk. XLV. p. 183.

102) Paulsen, O., 1908. "Peridiniales" in Brandt u. Apstein. "Nord. Plankt." No. 18.

103) Paulsen, O., 1911. Marine plankton from the East-Greenland Sea, III. Peridiniales. Danmark Expeditioentil Grönlands Nordöstkyst 1906-1908, III. No. 11.

104) Pavillard, J., 1907. Sur les *Ceratium* du Golfe du Lion. Bull. de la soc. bot. de France. LIV.

105) Pavillard, J., 1916. Recherches sur les peridiniens du Golfe du Lion. Trav. Inst.

Bot. Univ. Montpelier, series mixte. mem. No. IV. p. 9–70.

106) SCHILLING, A. T., 1891. Die Süsswasser-Peridineen. Flora III.

107) SCHILLER, J., 1911. Neue *Peridinium*-Arten aus der nordlichen Adria. Oes., Bot. Zs. Wien. LXI.

108) SCHMIDT, J., 1901. Peridiniales (Flora of Kohchang, pt. IV). Bot. Kidsskift XXIV.

109) SCHRÖDER, B., 1900. Das Phytoplankton des Golfes von Neapel. Mitt. a. d. Zool. Station z. Neapel. XIV.

110) SUHÜTT, F., 1887. Über die Sporenbildung mariner Peridineen. Ber. d. deutschen bot. Gesellsch. V.

111) SUHÜTT, F., 1892. Über Organizationsverhältnisse des Plasmaleibes der Peridineen. Sitzber. Akad. wiss. Berlin. 1892.

112) SUHÜTT, F., 1895. Die Peridineen der Plankton Expedition. Ergebnisse Plankton Expedition der Humboldt Stiftung. IV.

113) SUHÜTT, F., 1896. Gymnodiniaceae, Prorocentraceae, Peridiniaceae, Bacillariaceae. ENGLER u. PRANTL. Die naturlichen Pflanzenfamilien. I.

114) VANHÖFFEN, E., 1896. Das genus *Ceratium*. Zool. Anz. XIX.

115) VESENBERG-LUND, C., 1600. Von dem abhängigkeitsverhältnis zwischen dem Bau der Planktonorgánismen und dem spezifischen Gewicht des Süsswassers. Biol. Centralbl. XX.

116) WILLEMOES-SUHM, R. v., 1871. Zur Entwicklung eines Peridinium. Zeits. f. wiss. Zool. XXI.

117)*CLAPARÈDE, ED. et LACHMAN, J., 1858–1859. Etudes sur les infusoires et les rhizopodes. mem. de l'inst. nat. Génév. V.

118)*EHRENBERG, C. G., 1838. Dritter Beitrag zur Erkenntniss grosser Organisation in der Richtung des kleinsten Raumes. Abhandl. d. Berliner Akad. 1838.

119)*EHRENBERG, C. G., 1840. Beobachtungen von 274 Infusorienarten. Bericht über Verhandl. d. Berliner Akad. d. wiss. 1840.

120)*EHRENBERG, C. G., 1895. Über das Leuchten und über neue mikroskopische Leuchttiere des Mittelmeeres. Monatsber. d. Berliner Akad. d. wiss. 1895.

121)*GRAN, H. H., 1902. Das Plankton des norwegischen Nordmeeres. Rep. Norweg. Fisheries and Marine Invest. II. 5.

122)*LEVANDER, K. M., 1902. Eine neue farblose *Peridinium*-Art. Meddel. af Soc. pro Fauna et Flora fenica.

123)*OSTENFELD, C. H., 1903. Phytoplankton from the sea around the Faeröes. Bot. of the Faeröes II.

124)*OSTENFELD, C. H. og SCHMIAT, J., 1900. Plankton fra det röde Hav og Adenburgte. Vidensk. Meddel. fra den naturhist. Foren. i Kjøbenhvn.

125)*PAULSEN, O., 1907. The Peridiniales of the danish waters. Meddel. fra Kom. for Havundersögelser. ser. Plankton I. 1.

126)*STEIN, F. R., v., 1883. Der Organismus der Infusionstiere III, 2. Die Naturgeschichte der arthrodelen Flagellaten. Leipzig.